Genetic Analysis of Loose Smut (*Ustilago avenae* Pers. (Rostr.)) Resistance in Oat (*Avena sativa* L.)

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Abstract

Oat (*Avena sativa* L.) is a widely grown cereal crop that is used for human consumption and livestock feed. Canada is the second largest producer of oat worldwide, with Saskatchewan leading annual Canadian production with 1.7 million tonnes. As market prices for oat are usually lower than other cereals, growers tend to forgo the use of certified seed or crop inputs (e.g. seed treatment) when growing oat. This can have significant repercussions when it comes to diseases that affect both the quality and quantity of the crop. One such disease is loose smut of oat, caused by the basidiomycete pathogen *Ustilago avenae* (Pers.) Rostr. This study investigated loose smut resistance present in 'CDC Dancer' using an $F_{4:7}$ recombinant inbred line (RIL) population derived from the cross 'CDC Dancer' x 'AC Morgan' (DM). The goals were to: 1) phenotypically characterize, linkage map and identify putative marker(s) linked to loci governing resistance, and 2) to assess whether resistance from this population conferred a negative yield effect in the absence of the pathogen. A linkage map was constructed with data from single nucleotide polymorphism (SNP) genotyping of the population, in conjunction with both field and greenhouse the phenotypic disease assessments. The map was comprised of 34 linkage groups (LGs), with a major resistance gene mapping to the terminus of linkage group (LG) 11 and a minor resistance gene mapping to LG19. Investigation of allelic effects underlying each QTL revealed that lines possessing the 'CDC Dancer' allele at either QTL had a lower loose smut disease reaction score. Analysis of both QTL indicated an interaction ($P < 0.05$) in disease reaction scores. The markers linked to these two QTL will be useful to oat breeders wishing to incorporate this resistance into future oat cultivars. Evaluation of two genetically similar RILs from the DM population, which differed in their reaction to loose smut, indicated no difference in mean yield between the lines. As such, the absence of yield effects from the *U. avenae* resistance gene investigated in this study means that incorporation of this source of loose smut resistance is a viable option for oat breeders.

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List of Abbreviations

Abbreviation	Definition
AAFC	Agriculture and Agri-Food Canada
ABA	Abscisic acid
AC	Agriculture Canada
AFLPs	Amplified fragment length polymorphisms
AIs	Active ingredients
AM	AC Assiniboia x MN841801 F_7 population
ANOVA	Analysis of variance
APR	Adult plant resistance
CDC	Crop Development Centre (University of Saskatchewan, Saskatoon, SK)
cM	Centimorgans
CV	Coefficient of variance
CORE	Collaborative Oat Research Enterprise
DArTs	Diverse arrays technology
DF	Degrees of freedom
DM	CDC Dancer x AC Morgan $F_{4:7}$ derived recombinant inbred line population
DNA	Deoxyribonucleic acid
EST	Expressed sequence tag
ET	Ethylene
ETI	Effector-triggered immunity
FRAC	Fungicide resistance action committee
GxE	Genotype by environment interaction
GFG	Gene-for-gene
HR	Hypersensitive response
IL4	IL86-1156 x Clintland 64 $F_{5:8}$ population
JA	Jasmonic acid
KO	Kanota x Ogle oat population
LAI	Leaf area index
LG	Linkage group
LOD	Logarithm (base 10) of odds
MAS/MMAS	Marker-assisted selection/Molecular marker-assisted selection
MHPs	Monosomic hybrid plants
NILs	Near isogenic lines
OP	Otana x PI269616 F_6 population
OT	Ogle x TAMO-301 $F_{6:7}$ population
PB	Provena x CDC Boyer F_8 population
PG	Provena x 94197A1-9-2-2-5 F_8 population
PRCOB	Prairie recommending committee for oat and barley
QTL	Quantitative trait loci
RAPDs	Rapid amplified polymorphic DNA
RFLPs	Restriction-fragment length polymorphisms
RILs	Recombinant inbred line
R:S	Resistant to susceptible ratio
SA	Salicyclic acid
SCARs	Sequence characterized amplified regions

List of Abbreviations (Continued)

Abbreviation	Definition
SE	Standard error
SNP	Single nucleotide polymorphism
SSRs	Simple sequence repeats
TGW	Thousand grain weight
UPGMA	Unweighted Pair Group Method with Arithmetic Mean

1.0 Introduction

Common oat (*Avena sativa* L.) is a cereal crop grown worldwide that is produced mainly for human consumption and livestock feed. Second only to Russia, Canada produces 3.4 million tonnes of oat annually, grown on approximately 1.2 million hectares (Statistics Canada 2015, POGA 2016). Almost half of this production occurs in Saskatchewan where 550,000 to 700,000 hectares are seeded annually (Statistics Canada 2015). Oat has been praised for its nutrition profile with desirable levels of soluble β-glucan fibre, protein, fat, B vitamins, and other minerals (Sadiq Butt et al. 2008). Its characteristic β-glucan is associated with (positive effects on) control of diabetes, improved cardiovascular health, and reduced cholesterol, when consumed on a regular basis. Due to its lack of gluten protein, oat is also a viable option for those afflicted by celiac disease. Antioxidant properties have also been attributed to the avenanthramides, phytic acids, and vitamin E found in oat grain.

Ustilago avenae (Pers.) Rostr. is a basidiomycete pathogen that causes loose smut of oat. Disease symptoms occur in the panicles where the entire floret, consisting of ovary, palea, lemma and glumes, is replaced with dark brown to black powdery teliospores (or chlamydospores). Spores from infected plants are dispersed by wind or rain to uninfected oat flowers, where they germinate, forming a basidium that produces hyphae that eventually infects the ovary, and survives as dormant mycelium in the seed embryo (Agrios 2005; Parry 1990; Mills 9167). Spores that germinate in the flower may also remain as resting mycelia below the palea until the seed is sown (Sampson 1929). Alternatively, spores disseminated during harvest may remain on or below the hull of uninfected seed, and develop mycelia that infect the coleoptile and mesocotyl once the seed germinates (Western 1936). Loose smut exists wherever oat is grown and is capable of reducing yields by up to 10% (Parry 1990; Agrios 2005; Wang 2004).

One option to control loose smut is the use of systemic fungicidal seed treatments. However, seed treatment increases the potential for fungicide-tolerance to develop in the pathogen population and incurs a cost to growers. In addition, there are no seed treatment options available to control loose smut in organic systems. An alternative strategy to control loose smut is the use of resistant oat cultivars.

Previously effective *U. avenae* resistance, such as that possessed by the cultivar 'Victoria', is no longer effective as the widespread production of this cultivar increased selection pressure on the pathogen, causing it to develop virulence. The same occurred with resistance from the cultivar 'Clinton'. There have been no gene names attached to any source of resistance; they are only referred to based on the cultivar in which the resistance resides. While effective resistance currently exists, such as the 'Markton' type, nothing is known about the genetic control of resistance, the location of the gene(s) within the oat genome, the mechanism by which they provide resistance, or whether all currently resistant cultivars possess the same resistance gene(s).

Breeding for oat loose smut resistance is a time- and labour-intensive process. Disease evaluation must wait until full panicle emergence for symptoms to be displayed. Lines must be screened several times to confirm the presence of resistance because of the possibility of false negatives (disease 'escapes'). As a result, the overall process limits the number of lines that can be accurately assessed each year. Molecular marker-assisted selection (MMAS) is extremely useful for traits that are time-consuming or difficult to evaluate, such as loose smut resistance. MMAS would not only increase the efficiency of screening, but also the accuracy. To date there are no reports of molecular markers linked to any *U. avenae* resistance genes. In addition, markers linked to loose smut resistance would determine if resistant cultivars possess the same or different genes. In turn, this knowledge would be helpful to

prevent overuse of a single source of resistance that could increase selection pressure on the pathogen and lead to virulence development in the population.

Incorporation of a resistance gene into a given breeding line may have unexpected negative consequences on the line's agronomic performance. This effect may be the result of linkage drag, that is, genes tightly linked to the resistance gene that are introduced into a line, or the negative effect may be a result of the resistance gene itself. Understanding the impact of loose smut resistance on other important traits, such as yield, is valuable knowledge as it may influence the decision of a breeder to incorporate the gene, especially if alternative resistance sources exist or alternative control strategies are available.

The goal of this thesis was to address the limited knowledge related to loose smut resistance in oat, specifically, to determine the genetic control of resistance, the genomic location(s) of genes underlying currently effective resistance, and to understand the impact of loose smut resistance on oat yield.

1.1 Objectives and Hypotheses

The objectives of this thesis were:

(1) to assess the 'CDC Dancer' x 'AC Morgan' $F_{4:7}$ recombinant inbred line (RIL) population segregating for resistance to *U. avenae* in both field (mixed inoculum) and greenhouse conditions (using isolates A13, A60 and A617),

(2) to develop a genetic linkage map for the 'CDC Dancer' x 'AC Morgan' population, map loose smut resistance, and identify molecular markers linked to resistance, and

(3) to evaluate the effect of loose smut resistance on yield using lines from the 'CDC Dancer' x 'AC Morgan' lines that were very similar genetically, but differed in reaction to loose smut.

The hypotheses associated with these objectives were:

(1) the genetic resistance reaction to *U. avenae* in the 'CDC Dancer' x 'AC Morgan' population is not isolate specific,

(2) loose smut resistance is controlled by a single gene, and

(3) there is no effect of loose smut resistance on yield, in the absence of the pathogen and disease.

2.0 Literature Review

2.1 History of Common Oat

Common oat (*Avena sativa* L.) is an annual grass in the family Gramineae (Poaceae). The origin of oat is not clear, but the greatest areas of diversity can be found from the Canary Islands through the Mediterranean basin to the Middle East, with the centre of origin surmised to be within this area (Rines et al. 2006; Murphy and Hoffman 1992). Archaeological evidence points to regions of northern, western and central Europe, between 3000 to 4000 years ago, as the most likely place and time of domestication (Parry 1990; Ladizinsky 2012). Oat is often believed to have been domesticated as a 'secondary crop', that is, a weedy relative found within barley or emmer wheat fields, where individual plants with non-shattering grain characteristics were selected and subsequently grown as a separate crop (Thomas 1995, Rines et al. 2006). European colonists spread the crop to North America, as well as Australia and New Zealand (Rines et al. 2006). Today, countries with the largest production of common oat are, in descending order, Russia, Canada, Poland, Australia, Finland and the United States, with significant production in China, Argentina and several European countries (FAOSTAT 2016).

Members of the genus *Avena* have a basic chromosome number of seven, and exist as diploid, tetraploid and hexaploid species. The diploid *A. strigosa* and the tetraploid *A. abyssinica* are the only non-hexaploid species cultivated, but not in any significant amount, the former grown as a forage crop in Europe and South America and known as 'black oats' in Brazil (Rines et al. 2006). *Avena abyssinica* is used to a limited extent as a food crop in Ethiopia. The most widely cultivated oat species is common oat, with *A. byzantina* C. Koch., referred to as red oat, grown to a lesser degree. Both species are allohexaploids. Two wild oat species, *A. fatua* L. and *A. sterilis* L., are also hexaploids and readily cross with the cultivated species. This can be problematic as both species are weeds within common oat crops, which limits chemical control options. However, *A. sterilis* has been an extremely useful source of genes, specifically those related to crown rust (*Puccinia coronata* Corda f. sp. *avenae* Eriks) resistance.

The *A. sativa* hexaploid (AACCDD) genome is thought to have been formed from three diploid progenitors, each containing seven chromosomes, to produce a 2n=6x=42 genome. Recent studies on the genetic origin of the hexaploid genome have led to the conclusion that there were multiple polyploidization events, involving both diploid and tetraploid ancestors that gave rise to the genome traditionally known as AACCDD (Chew et al. 2016). Genotype-by-sequencing (GBS)-based clustering of a diverse set of *Avena* species comprising different ploidy levels grouped together *Avena longiglumis*, *A. canariensis*, and *A. wiestii*, which represented the progenitor species of the A genome (Chew et al. 2016). From these findings it was postulated that fusion of one of the A genome accessions with either *A. clauda* or *A. eriantha* providing the C genome, led to the AACC tetraploid (Chew et al. 2016). The closest related species to the tetraploid ancestor that currently exists are believed to be *A. insularis*, *A. magna* (also referred to as *A. maroccana Gdgr.*), and *A. murphyi* (Chew et al. 2016). It was also postulated that a second diploid from the A cluster was then involved in creation of the current AACCDD hexaploid upon fusion with the tetraploid AACC (i.e. after fusion of the genomes this second AA genome diverged over time to become the DD sub-genome). It was suggested that the A cluster diploid involved in the creation of the hexaploid was not the same species as the A cluster diploid involved in the creation of the tetraploid given that there is variation between the A-derived genomes, typically referred to as A and D (Linares et al. 1998; Sanz et al. 2010). It was previously suggested, based on the absence of a current diploid species with a D genome, that the D genome may be a duplication of the A genome, given its similarity (Thomas 1995; Rines et al. 2006). Both *A. fatua* and *A. sterilis* are believed to be the intermediate ancestors of *A. sativa*. *Avena fatua* appears to have contributed the mutation conferring *A.*

sativa's retention of grain, while the wide distribution of *A. sterilis* and its ecological adaptability demonstrates its likely role in the dissemination of hexaploid oats to temperate climates (Thomas 1995).

The first record of oat grown in Canada was in 1617 in what is now Quebec (Grant 1939). Oat cultivation spread to the Canadian Prairies by the mid-1800's, and was established as the third largest cereal crop, with uses as both forage and grain. Oat is well suited to the climate of Western Canada, especially the black soil zone where cool, moist conditions allow for good grain development and growth throughout the entire season (Rines et al. 2006). Despite the suitability of the crop, oat acreage has declined, initially due to the decline in the use of horses as a source of farm labour, but later as a result of the availability of higher value crop options and other high protein livestock feed, specifically in the United States dairy industry (Rines et al. 2006). Despite these changes, Canadian oat production in 2015 was 3.4 million tonnes (Statistics Canada 2015). Estimates of area sown to oat in 2015 was 1.3 million hectares, with approximately half of the production occurring in Saskatchewan (690,000 hectares), followed by Alberta and Manitoba with 270,000 and 190,000 hectares, respectively (Statistics Canada 2015).

A major use of oat worldwide is for livestock feed, as pasture, hay, silage, grain, and straw bedding. In some areas of the world, such as South America, oat is a large part of the human diet. Oat has been touted for its many nutritive characteristics, including high beta-glucan levels (fibre), good quality and quantity of protein in the kernel, desirable amino acid profile, a favourable ratio of unsaturated fatty acids, and the presence of antioxidants, such as tocols and avenanthramides (Rines et al. 2006). It is also considered to be a hardy crop, more tolerant ofadverse weather and soil conditions, and water logging than other crops commonly grown in similar regions, such as barley or wheat, and generally less affected by diseases.

2.2 *Ustilago avenae* (Loose Smut) Significance

There are approximately 1,200 smut species affecting plants worldwide. Loose smut is a seedborne disease of oat, caused by the pathogen *Ustilago avenae* (Pers.) Rostr. A related oat pathogen, *U. kolleri* Wille, causal agent of oat covered smut, can be distinguished morphologically from *U. avenae* as it has smooth (non-echinulate) spore walls, while *U. avenae* has echinulate walls (Harder and Haber 1992). Hybridization between the two species does occur and thus their genetic relationship is not fully understood (Harder and Haber 1992; Huang and Nielsen 1984).

Loose smut is commonly found wherever oat is grown. Grain yield losses due to smut were a large issue prior to the 1900s, with the first significant record of oat smut reported in 1894 at the Brandon Experimental Farm in Manitoba (Estey 1994; Agrios 2005). In 1896, oat yield losses in Manitoba and Saskatchewan due to smut reached levels of 10-25% and it was considered a serious cereal disease in the Prairies (Johnson 1961). Today, yield losses between 10-40% due to smut are possible due to smut, but generally losses are 1% or less in both the UK and USA, and around 7-10% in China (Parry 1990; Agrios 2005; Wang 2004). The disease can still increase rapidly if certified seed, seed treatments, or resistant cultivars are not used. Continuing to develop and maintain smut resistance in cultivars is therefore important, especially when oat is produced under organic conditions where seed treatments are not an option (Thomas and Menzies 1997).

Infected plants have a direct effect on yield, and the spread of the sooty smut spores among harvested seed significantly decreases the quality of the grain, as well as affecting seed for subsequent planting (Agrios 2005). Seed sown from a previous crop infected by smut results in increasing smut levels in subsequent crops (Menzies et al. 2009). Guidelines governing the amount of true loose smut allowed in barley for each grade have been established, however no such guidelines exist in oat (Seeds Regulations 1.01, 1.1).

2.3 Control of *Ustilago avenae*

2.3.1 Genetic Control

Loose smut of oat is considered a seedling disease, as the pathogen infects plant tissue once germination occurs. The pathogen comes in contact with the seed either during flowering, when spores from infected plants are blown onto florets of healthy plants, or through contamination of mature seed during harvest. It is unknown whether the resistance mechanisms occur immediately, such as preventing pathogen penetration of the pericarp, or at some later point during seedling development. In resistant wheat and barley varieties, Popp (1951) observed that embryos were not infected by true loose smut pathogens, but were in susceptible varieties. The interaction between a specific cultivar of wheat or barley and physiological race of smut determined whether embryos were infected or not. These observations suggested that specific genes within the host and pathogen were critical to the development of compatible or incompatible reactions. Willits and Sherwood (1999) detected the covered smut pathogen, *U. hordei*, in the leaves of resistant barley cultivars, which supported previous findings by Groth and Person (1978). This illustrated that the pathogen was able to infect embryos of resistant plants, but progression was limited at some later point in the plant's lifecycle such that the heads were unaffected and symptomless (Willits and Sherwood 1999).

Gabor and Thomas (1987) identified both seed and seedling resistance to true loose smut (*Ustilago nuda)* of barley. In barley cultivars with seed resistance, hyphae were detected in only 3% of inoculated seed, suggesting the resistance mechanism occurred prior to infection of the embryo, thus preventing hyphal growth. Seedling resistance occurred in other barley cultivars, where the embryos in 50% of the seeds were infected, but none of the mature plants had smut symptoms. In other cases, barley loose smut infection halts main shoot growth, but development of healthy tillers are observed later (Mumford and Rasmusson 1963).

While loose smut can be controlled by fungicidal seed treatment, this practice is not permissible for organic growers, nor is it desired by conventional growers. Although hot water treatments can be effective for controlling smut on contaminated seed, and this is an option for organic growers, it is not necessarily feasible for large amounts of seed. For many conventional growers who view oat as a low input (low risk) crop, it is not economically desirable to use inputs on the seed. Thus, a requirement of treated oat seed might cause growers to grow another crop. As well, fungicide use to protect oat from smut is not necessarily possible in all growing areas worldwide, due to high costs, lack of equipment, or knowledge of treating seed.

Resistant cultivars are the best option for managing this disease, as there is less cost, less environmental impact, and they provide a control option for all producers. Resistance to smut is a Priority 1 disease consideration for potential new lines for cultivar registration by the Prairie Grain Development Committee in Canada (PRCOB 2015). Continuous investigation of new sources of resistance is important to provide potentially effective resistance in the event that new *U. avenae* races evolve, as has been observed in the past.

The development of new loose smut races is continuous as both mutation and meiosis occur within the extremely large number of teliospores produced during every cycle of the disease (Agrios 2005). For example, the oat cultivar 'Victoria' was introduced to North America in 1927 as it was resistant to crown rust (*Puccinia coronate* f. sp. *avenae*), which was also a significant disease issue at the time (Menzies and Thomas 1997; Reed and Stanton 1942). 'Victoria' was subsequently determined to be resistant to loose

smut. Due to disease resistance and thus reduced yield loss, the cultivar was widely grown across North America, including the Canadian Prairies. Cultivar monoculture unsurprisingly led to development of *U. avenae* isolates that overcame the resistance present in 'Victoria'. The first instance of *U. avenae* with virulence to 'Victoria' was identified in 1934 from an isolate collected in Oklahoma (Reed and Stanton 1942). However, virulent isolates were not found in Canada (namely Manitoba and Saskatchewan) until 1969 (Nielsen 1972; McDonald et al. 1971).

2.3.1.1 Gene-for-Gene Theory

The gene-for-gene concept was originally proposed by Flor (1942) after investigating the reaction of the rust pathogen *Melampsora lini* on flax. He described the interaction between plants and pathogens, or host and parasite, based on patterns of host resistance and pathogen virulence governed by single genes, also known as vertical resistance as termed by Van der Plank. The basis of the theory is commonly summarized as "For each gene for resistance in the host, there is a corresponding gene for avirulence in the parasite" (Kerr 1987).

The gene-for-gene concept in relation to *A. sativa* and the loose smut pathogen *U. avenae* was first demonstrated by Holton and Halisky (1960). Their experiments involving the differential oat cultivars 'Anthony', 'Gothland', 'Monarch' and 'Camas' supported monogenic, or single gene, resistance to loose smut (Holton and Halisky 1960). Since that time, one, two and three gene control of resistance to loose smut has been hypothesized in different cultivars. The cultivar 'Victoria' was thought to contain one or two dominant resistance genes (Cherewick and McKenzie 1969). *Ustilago avenae* virulent against the resistant gene(s) in 'Victoria' developed by 1969 and became widespread throughout Canada (Nielsen, 1972). 'Markton', derived from a Turkish line, is believed to carry two or three genes for resistance to loose smut (Reed and Stanton 1938; Holton and Murphy 1966). 'Camas', which arose from a cross between 'Markton' and 'Victory', carries a single gene for *U. avenae* that also confers resistance to *U. kolleri*, the covered smut pathogen of oat (Cherewick and McKenzie 1969). 'Black Mesdag' and 'Fulghum' are cultivars that contain single resistance genes that differ from one another (Reed 1925, 1935). However, *U. avenae* virulence to 'Black Mesdag' and 'Fulghum' was identified in Minnesota by 1990 (Wilcoxson and Stuthman 1993).

A host differential set has been established to screen isolates of *U. avenae* and determine their virulence spectrum. This series includes the cultivars 'Anthony', 'Black Diamond', 'Victory', 'Gothland', 'Monarch', 'Camas', 'Black Mesdag', 'Atlantic', 'Fulghum', 'Clintland', 'Nicol', IL79-4924, CI5575, and 'Markton' (Menzies and Thomas 1997). While these cultivars all possess resistance, there is a poor understanding of the allelic relationships and genomic locations of these genes. Additionally, within the current elite oat gene pool there is a poor understanding of which resistance genes are prevalent. As such, defining oat smut resistance genes, in terms of genomic location and effectiveness, within the historical and cultivated oat gene pool will help establish a foundation of knowledge against which new resistance genes may be compared and characterized for their potential value. This knowledge will also be beneficial for the process of incorporating these genes into new varieties.

2.3.2 Chemical Control

Chemical seed treatments that inhibit infection by *U. avenae* must be systemic to prevent seedling penetration of the fungus. Historically, formalin (formaldehyde in solution) was used as a seed treatment to prevent loose smut infection. Today, formalin has been replaced with a variety of crop protection products. Active ingredients (AIs) in fungicidal seed treatments that control loose smut of oat include

difenoconazole, triadimenol, triticonazole, tebuconazole and ipconazole (all FRAC Group 3), metalaxyl-M (mefenoxam, Group 4), carbathiin/carboxin (Group 7), and thiram (Group M3) (OMAFRA staff 2011; Thomson and Ockey 1998; FRAC 2016). However, the use of some of these ingredients poses a high risk for development of fungicide insensitivity in *U. avenae*. Isolates of the related pathogen *U. nuda* (Jens.) Rostr., causing loose smut in barley, that are resistant to the Group 7 carboxamide, fungicidal ingredient carboxin, were identified as early as 1986 in France and have been found more recently in Italy (Leroux and Berthier 1988). Carboxin-tolerant isolates of this pathogen, as well as *U. tritici* (Pers.) Rostr. which causes loose smut in wheat, have also been found in Canada (Menzies 2008). Thus, it is possible that *U. avenae*, with a similar biology to the smuts of barley and wheat, could develop resistance to the same fungicide modes of action. Resistance of *U. avenae* to Group 3 fungicides has already been detected in a laboratory setting (Hippe and Koller 1986). Thiram, a Group M3 dithiocarbamate, is the only active ingredient considered to pose a low risk of resistance development because it targets the pathogen at multiple sites (Fishel and Dewdney 2006). Despite differing risk levels for resistance development, any fungicide use can increase the potential of insensitive pathogen populations developing. The same can be said for genetic control of the pathogen, however, multiple sources of resistance can provide protection to the crop without any added costs to the grower, or risk of exhausting chemical protection.

2.4 Disease Epidemiology

Ustilago avenae is an obligate parasite (or biotrophic pathogen) that belongs to the family Ustilaginaceae within the phylum Basidiomycota, more commonly referred to as Basidiomycete fungi (Hyde 1972; Ellis et al. 2007). Basidiomycete fungi are distinguished on the basis of their sexual spore-producing structure, called a basidium, which is club-like in shape and produces sexual basidiospores (Agrios 2005).

Ustilago avenae overwinters as dormant mycelia in the embryo (in the scutellum) of an infected oat caryopsis or between the kernel and the hull (Agrios 2005). Once germination occurs, the mycelia initially grow intracellularly in the seedling after which the mycelia begin to grow intercellularly following the growing point of the plant (Agrios 2005; Parry 1990, Mills 1967; Western 1936). Mycelia invade the floral initials, and instead of seed development, teliospores (also called chlamydospores) of the fungus develop. The teliospores replace all floral tissues with the exception of the rachis. Upon reaching maturity, infected tillers and plants are generally shorter than healthy plants. Teliospores are subsequently dispersed to healthy flowers on nearby plants by both wind and rain, where they germinate. A basidium is formed following germination, and it then produces haploid hyphae, or promycelia. Compatible hyphae undergo fusion creating dikaryotic hyphae that infect the ovary by growing through the stigma or ovary walls (Agrios 2005; Parry 1990; Nyvall 1999). This mycelium becomes dormant in the embryo until the seed germinates, thus no disease symptoms are present on seed contaminated through spore dispersal during the growing season. Harvest activity can also cause spores to lodge on or beneath the hulls of healthy seed, which are then able to infect once the seed germinates (Nyvall 1999). The loose smut disease is monocyclic.

2.4. 1 Conditions Favouring Disease Development

Loose smut of oat is found worldwide, but appears to favour humid or sub-humid areas, as these conditions during anthesis may prolong flowering (Agrios 2005; Parry 1990). Mycelial infection occurs when kernels germinate at soil temperatures between 5^{o}C to 30^{o}C and soil moisture levels from 5% to 60%, with optimum conditions at 15-25^{o}C and relatively dry soils at 35-40% moisture (Harder and Haber 1992; Nyvall 1999). Neutral and slightly acidic soils appear to support the infection process of the pathogen (University of Illinois Extension 1988).

2.4.2 Symptoms of Loose Smut Infection

Symptoms of loose smut are not usually found until panicle emergence, with diseased plants potentially emerging earlier, and for a slightly shorter duration, than uninfected plants (Agrios 2005; Harder and Haber 1992). Upon emergence, the smutted panicle tends to spread less than uninfected panicles and typically all grain within the smutted panicle, including glumes and awns, is replaced by teliospores (Nyvall 1999). Every floret and panicle on infected oat plants are typically ‘smutted’ (Nyvall 1999). In contrast, wheat bunt caused by *Tilletia caries* has been known to infect specific tillers, with older tillers often smut-free (Swinburne 1963). Similarly, barley infected with *U. hordei* or *U. nuda*, typically has older tillers that are smut-free (Faris 1924; Beattie 2014, per. comm.). The membranes enclosing the teliospore mass on the oat plant eventually burst, releasing the teliospores and leaving the rachis bare (Agrios 2005). Aside from visual observation of teliospores on or within the hull of oat kernels, contaminated seed can only be detected by staining of the excised embryo to detect mycelia (Parry 1990).

2.5 Genetic Mapping

Genetic mapping is the process by which markers are associated with a specific trait and their position(s) located along a genetic linkage or physical map. If a marker has been physically assigned to a chromosome, then this information can also be assigned to the trait controlled by the associated gene(s). Genetic control of a trait can be as simple as a single gene (typically a qualitative trait) or by many genes (typically a quantitative trait). Phenotypic data is collected for a trait segregating in a population developed from a bi-parental cross (e.g. recombinant inbred population (RIL) or F2 population), or in a set of unrelated lines (association mapping). Thousands of molecular markers are screened in the lines making up the population. Currently, single nucleotide polymorphisms (SNPs) assayed through genotype-by-sequencing (GBS), or using platforms such as the Illumina GoldenGate or Infinium assay, are used to generate the genotypic data that is used to construct linkage maps. Maps are often compared with previously constructed maps, when available, to check marker order placement, and mapping distances. The genetic information collected in populations is then used in combination with the phenotypic data to identify genes or loci controlling the trait.

2.6 Marker-Assisted Selection in Oat Breeding

Markers identify sources of DNA variation that when closely linked to genes, allow for selection based on genotype rather than phenotype. This type of selection is often referred to as molecular marker-assisted selection (MMAS) or marker-assisted selection (MAS). A characteristic controlled by single genes, such as some disease resistances, can be effectively selected with markers, while traits controlled by multiple genes, quantitative trait loci (QTL), can also be selected, but genetic gain is slower due to the quantitative nature of these traits. The ultimate molecular marker position is within a gene (also known as a perfect marker), thus the goal is always to locate a perfect marker or a marker tightly linked to the gene of interest to minimize the chance of recombination occurring between the marker and gene. Molecular markers such as restriction-fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), random amplified polymorphic DNA (RAPDs), diverse arrays technology (DArTs) and sequence characterized amplified regions (SCARs) have been used in oat breeding to detect genes that control various traits. However, among these, only RFLPs are co-dominant in nature, which allows identification of heterozygotes, and none are suitable for high throughput genotyping for specific traits (Gnanesh et al. 2013).

Single nucleotide polymorphisms (SNPs) are the current molecular marker of choice for genetic mapping. The SNPs, located in both genic and non-genic regions, are allele-discriminatory and are highly abundant within most plant genomes. They are also amenable to automated high-throughput screening methods. Until recently, the number of molecular markers available for oat researchers and breeders was low compared to other species, in part due to the complexity of the hexaploid genome. Polyploid genomes, such as oat, present challenges in mapping markers as regional sequence and gene duplication in both homologous and homoelogous chromosomes can cause markers to be mapped to multiple loci within the genome (Akhunov et al. 2003; Blanc et al. 2000; Portyanko et al. 2001; Wight et al. 2003). As a result, MMAS has been used to a limited degree in oat breeding, with markers linked to *Pc91*, *PcKM* and APR crown rust resistance genes the exception (Gnanesh et al. 2013; Gnanesh et al. 2015; Lin et al. 2014).

Recently the Collaborative Oat Research Enterprise (CORE), a consortium of oat researchers from North American, Australia, South America and Europe, addressed the lack of genetic tools available to the community. The result of their work was the creation of a more saturated, expressed sequence tag (EST)-based SNP genetic linkage map that was physically anchored to all 21 oat chromosomes (Oliver et al. 2013; Chaffin et al. 2016). In addition, a 6K Infinium SNP genotyping assay was created that permitted access to the SNPs identified by the CORE group. This genotyping tool is useful for identifying new markers linked to traits, such as loose smut resistance. Markers linked to this trait would represent an improvement in the evaluation of the trait since traditional screening in the field or greenhouse is time, cost, and labour intensive. There are also issues with maintaining inoculum, ensuring inoculation is effective, and determining if symptomless plants are escapes.

Initial work by Eckstein et al. (2002) and Kibite et al. (2004) identified a co-dominant SCAR marker that was linked (≈ 5 cM) to a gene conferring resistance to *U. avenae* isolate A13. This marker was also within mapping distance to resistance genes specific to A617 and A60, at 8 cM and 18 cM, respectively (Kibite et al. 2004). However, the inability to convert this marker to high-throughput screening systems, such as TaqMAn or KASP assays, and it's fairly large genetic distance from the resistance gene(s) has limited its utility. It is unknown whether the resistance in the 'CDC Dancer' x 'AC Morgan' population is also effective against all three isolates and if it is a single gene or several closely linked genes.

2.7 Impacts of Incorporating Resistance Genes

A significant priority of plant breeding is improving crop yield. It is no surprise that pathogens causing disease, as well as other pests of crops, detrimentally influence yield. While cultural and chemical practices can aid in decreasing the impact of these problems, inherent resistance within the plant is highly sought to reduce the inputs required to produce a successful crop. One or more resistance genes within a cultivar are effective in maintaining yield when the pathogen or pest is present, and will result in greater yield than cultivars lacking resistance. However, it is when the pathogen or pest is absent that the impact on yield by resistance genes is not fully understood. As yield is known to be a multi-genic trait, there are numerous ways in which it can be influenced.

Resistance genes are often discovered in wild relatives of domesticated crop species. While breeding advancements have improved the ease by which resistance genes can be incorporated into breeding lines, there is still difficulty ensuring that only the gene of interest is transferred. Genes associated with low yield that are linked to the resistance gene (i.e. linkage drag) can negatively impact yield in the adapted parent background (Yuan et al. 2002). Alternatively, the resistance gene itself can interfere with processes that influence yield. Current research is bringing to light the many ways in which defence pathways and growth and development pathways are intertwined. Resistance genes can influence both networks and have downstream pleiotropic effects that impact plant processes that affect yield (Brown and Rant 2013). Signalling defence/immune hormones such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) interact both negatively and positively with plant growth hormones, such as gibberellins (GA), cytokinins (CK), auxins, abscisic acid (ABA) and brassinosteroids (Denance et al. 2013). Some constitutive defence mutants have increased levels of defence hormones, such as SA, which plays a role in resistance to biotrophic and hemi-biotrophic fungi, or JA, which influences resistance to necrotrophic fungi (Robert-Seilaniantz et al. 2013). These elevated defence hormones interact with growth hormones by altering their regulation, resulting in developmental differences in the plant, which in some cases can affect yield. For example, SA has been linked to limiting growth by inhibiting auxin signalling (Huot et al. 2014). The same growth influence has been illustrated with SA signalling impeding GA signalling (Gallego-Giraldo et al. 2011). Auxins are involved with root and shoot elongation, while GA is involved with flowering and seed development. Inhibiting such phytohormones no doubt has repercussions on plant development, and in turn, on yield. Likewise, JA has been shown to interfere with the auxin signalling pathway, as well as the GA pathway (Wasternack and Hause 2013; Heinrich et al. 2013).

Several studies have analysed the relationship between disease or pest resistance and agronomic traits, including yield, in crop species. Resistance to powdery mildew in barley, leaf rust in winter and spring wheat, soybean cyst nematode and sudden death syndrome in soybean, have all shown decreased grain yield when grown in disease-free conditions in comparison to parent lines lacking resistance (Smedegaard-Petersen and Stolen 1981, Ortelli et al. 1996, Singh and Huerta-Espino 1997, Kabelka et al. 2006, Rupe et al. 1993). In contrast, studies involving crown rust resistance in oat and soybean cyst nematode studies in soybean have found no detrimental effect on yield, or a yield increase when resistant lines were compared to parent lines or near isogenic lines without the gene of interest (Frey and Browning 1971, Yuan et al. 2002). These varying results with soybean cyst nematode resistance indicate that not all resistance loci have a similar effect on yield in the absence of disease pressure, and thus each source of resistance for a given disease warrants investigation into its effect on yield.

3.0 Elucidation of Loose Smut (*Ustilago avenae*) Resistance Loci in *Avena sativa* L.

3.1 Introduction

Cultivated oat, *Avena sativa* L., is an important cereal crop grown worldwide for both food and livestock feed. Oat is considered a healthy cereal due to a number of nutritional compounds found within the grain, including β-glucan. β-glucan is a soluble fiber that has been demonstrated to lower plasma cholesterol and reduce the risk of heart disease in numerous studies (Queenan et al., 2007; Liatisa et al., 2009). This has resulted in health claims being established in both Canada (Health Canada, 2010) and the United States (U.S. FDA, 1997). Oat grain also contains a number of antioxidant compounds, including the polyphenolic avenanthramides, which have been shown to have an anti-inflammatory effect that may protect against coronary heart disease (Meydani 2009). In addition, oat contains 12–20 % high quality protein and low fat content (<8 %). Total annual world production of oat over the past five years has ranged from 21 to 24 million metric tonnes (FAOSTAT 2016) with the top three producing countries being Russia, Canada and Poland, producing an average of 4.5 million, 3.0 million, and 1.4 million metric tonnes, respectively (FAOSTAT 2016).

Production and quality of oat can be impacted by several diseases including loose smut. Loose smut is a seed-borne disease caused by the biotrophic basidiomycete pathogen *Ustilago avenae* Pers. Rostr. (Hyde 1972; Ellis et al. 2007). This disease has a direct effect on yield as infected grain is replaced with sooty-like teliospores (Green et al. 1968). Teliospores are dispersed during harvest and by rainy or windy conditions to uninfected oat panicles. The pathogen overwinters as dormant mycelia in the scutellum or below the hull of infected oat seed. Following seed germination in the spring, fungal mycelia grow intracellularly within the seedling after which the mycelia begin to grow intercellularly behind the growing point of the plant (Agrios 2005; Parry 1990). An infected oat plant at maturity appears stunted in comparison to uninfected plants and once flowering commences, mycelia grow into all florets causing damage to all tissues except the rachis. Mycelia eventually invade the developing ovary and fill the seed with dark brown to black teliospores (also called chlamydospores) (Agrios 2005; Parry 1990). In Canada, the presence of oat loose smut was first recorded in Manitoba in 1894 (Estey 1994; Agrios 2005). While yield losses have reached 10-40% in the past, current losses of 1-10% are typical due to a range of effective control methods (Johnson 1961; Parry 1990; Agrios 2005; Wang 2004).

Resistant cultivars, certified seed, and systemic seed treatments all contribute to reducing the prevalence of loose smut. However, oat is considered a low-input crop and growers often do not invest in seed treatments or purchase certified seed from year to year, and instead choose to plant seed from previous harvests. Organic production systems do not permit the use of synthetic fungicides and there is currently no natural alternative to systemic seed treatments available in Canada. However, the biological fungicide *Pseudomonas chlororaphis* is available in Europe (under the trade names of Cedemon™ and Cerall™) to combat loose smut, but this product is not yet obtainable in Canada (Ozer and Coskuntuna 2016). Fungicidal seed treatments containing active ingredients from FRAC Groups 3, 4, 7, 11, and M3 are effective at controlling oat loose smut. However, these fungicide groups have varying levels of risk associated with them for the development of fungicide-tolerant pathogen populations. For example, carboxin and fenfuram (Group 7) resistant isolates of the pathogens causing loose smut in barley and wheat, *U. nuda* (Jens) Rostr. and *U. tritici* (Pers.) Rostr, have already been identified in France, Italy, and Canada (Menzies 2008). Due to the similar biology of oat loose smut to barley and wheat loose smut, *U. avenae* could develop resistant populations to the same fungicides or modes of action. While genetic control also acts as a selection pressure, creating a risk for virulent isolates to develop, multiple sources of resistance can provide protection to the crop without any added costs to the grower, or risk of

exhausting chemical protection. Thus, genetic resistance within cultivars is the most desired method of loose smut management because it is economical, effective and can be used in all production systems.

Genetic resistance to loose smut has been reported in North American oat cultivars that date back to the 1920's with resistance typically being governed by one or two dominant genes. For example, 'Victoria' a Uruguayan line introduced to North American in the 1920's, was reported to carry one or two dominant resistance genes (Cherewich and McKenzie, 1969). 'Markton' which was developed at Moro, Oregon in the early 1920s by selection from a Turkish introduction was reported to carry two resistance genes which control *U. avenae* (Reed and Stanton 1938). The resistance genes in these two cultivars are likely different as widespread virulence to the 'Victoria' resistance has been reported since 1969 in Western Canada (Nielson 1972). Two other cultivars, 'Black Mesdag' and 'Fulghum', have also been reported to carry single dominant resistance genes which differed from one another (Reed 1925, 1935). Virulence was reported on these cultivars based on surveys conducted from the late 1970's to 1990 in Minnesota (Wilcoxan and Stuthman 1993). Effective genetic resistance to loose smut has been incorporated into oat cultivars currently available to oat producers in Western Canada. The genetic control and genomic location of the resistance gene(s) currently used is unknown, however, 'Markton' is one likely source of resistance as this cultivar was used extensively in Western Canadian breeding programs since the 1970s (Menzies and Thomas 1997).

Breeding for loose smut resistance is an important component in oat breeding programs however, it is a time- and labour-intensive process. Disease evaluation must wait until full panicle emergence and because of the possibility of false negatives (disease 'escapes') lines must be screened several times to ensure the presence of resistance. As a result, the overall process is inefficient and could be improved greatly by molecular marker-assisted selection (MMAS). Molecular marker-assisted selection would allow for more efficient selection of loose smut resistance, and the development of such markers will assist in identification of the genomic location of resistance genes, such that different resistance sources could be characterized relative to one another.

Progress on the use of MMAS with oat has been slow due to the complexity of the oat genome and limited availability of useful molecular markers. The allohexaploid genome of oat (AACCDD) has a substantial portion of repetitive sequences and widespread gene duplication on multiple chromosomes (Flavell et al. 1977; Portyanko et al. 2001). These characteristics have made the creation of genetic linkage maps and the development of molecular markers linked to traits a challenge due to the tendency of markers to map to multiple locations within the genome (Portyanko et al. 2001; Wight et al. 2003).

Recently the Collaborative Oat Research Enterprise (CORE), a consortium of oat researchers from North American, Australia, South America and Europe, addressed the lack of genetic tools available to the community. The result of their work was the creation of a well saturated, expressed sequence tag (EST)-based SNP genetic linkage map that was physically anchored to all 21 oat chromosomes (Oliver et al. 2011; Oliver et al. 2013; Chaffin et al. 2016). In addition, a 6K iSELECT Infinium SNP Assay was created which permitted access to the SNPs identified by the CORE group. This genotyping tool already has demonstrated its usefulness for identifying markers linked to traits such as the oat crown rust resistance genes *PcKM* (Gnanesh et al. 2015) and adult plant resistance genes (Lin et al. 2014).

Initial work to define the genomic location and develop molecular markers linked to loose smut resistance in Western Canadian oat germplasm was conducted by Eckstein et al. (2002) and Kibite et al. (2004). Using three different isolates which represented the prevalent races found in Western Canada, it was determined that three clustered isolate-specific genes controlled resistance to these isolates. A

SCAR marker (Ua300) was developed and found to be linked from 5-18 cM from the three resistance genes which were located on linkage group KO 14 in the Kanota x Ogle mapping population (Wight et al. 2003, Tinker et al. 2009). However, the large recombination distance and the inability to convert the Ua300 marker to a high-throughput genotyping assay, such as TaqMan or KASP, limited the usefulness of the marker.

The purpose of this project was to understand the genetic control of loose smut resistance derived from 'CDC Dancer' germplasm and to genetically map the location of the resistance gene(s). The objectives were to: (1) to assess resistance to *U. avenae* derived from 'CDC Dancer' in both field (mixed inoculum) and greenhouse conditions (individual isolates) and determine if the resistance is effective in all cases, (2) to use the recently developed Oat 6K iSELECT Infinium SNP Assay to genotype a population segregating for loose smut resistance and identify markers linked to the resistance gene(s). It was hypothesized that the resistance reaction to the *U. avenae* pathogen in the 'CDC Dancer' x 'AC Morgan' population is not affected by isolate and that the resistance is controlled by a single gene.

3.2 Materials and Methods

3.2.1 Plant Material

An oat population derived from the cross 'CDC Dancer' x 'AC Morgan' (DM) was used throughout the study. The population was comprised of 160 $F_{4:7}$ recombinant inbred lines (RILs) formed by bulking the population until the F_4 generation at which point single panicles were selected to create individual lines, which were carried forward to the F_7 generation. 'CDC Dancer' is resistant to loose smut of oat, while 'AC Morgan' is susceptible.

'AC Morgan' was registered in 2000 and was developed from the cross OT526 x OT763 by the Lacombe Research Centre, Agriculture and Agri-Food Canada (Lacombe, Alberta) (Kibite and Menzies 2001). It is a medium maturing cultivar with high yield and desirable grain features including high protein, low oil, low hull percentage, and plump kernels. AC Morgan is susceptible to various diseases, including loose smut of oat. 'CDC Dancer' was derived from the cross OT344 x OT269 (= W90279) and was registered in 2000 by the Crop Development Centre (Saskatoon, Saskatchewan) (Canadian Food Inspection Agency 2017). It is a medium maturing cultivar with lower yield than 'AC Morgan', but it has excellent grain and milling quality as well as possessing resistance to loose smut.

All greenhouse and field trials included the parent lines 'CDC Dancer' and 'AC Morgan', as well as, check lines 'Starter' (resistant), 'Ogle' (susceptible), 'Belle' (susceptible), 'Hazel' (susceptible), and PY11108 (susceptible).

3.2.2 Pathogen Inoculation

Oat seeds were placed in glass test tubes and covered with an excess of inoculum solution which consisted of 10 g *U. avenae* teliospores per 1 L of tap water containing 0.67% Tween® 20. The glass test tubes were fitted with perforated stainless steel caps and placed in a metal basket within a glass desiccator. A vacuum of negative 138 kPa was applied to the desiccator for 3 minutes and then released. This vacuum procedure was repeated twice at which point each test tube was emptied onto a sieve to remove the inoculum solution. The sieved seeds were then placed on absorbent laboratory bench paper to dry for a minimum of 24 h. Seeds were planted within one week of inoculation. Teliospores for subsequent inoculations were obtained by collecting smutted oat panicles after each round of disease evaluation. Panicles were dried, ground with an Oster blender and the resulting smut-plant tissue powder shaken and pressed through two metal sieves with openings of 250 µm and 125 µm. This allowed the plant material to be almost completely removed from the *U. avenae* teliospores.

Inoculum used in the greenhouse disease screening trials (described below) consisted of individual loose smut isolates. Three different loose smut isolates (A13, A60 and A617), originally collected from Western Canada and provided by Dr. James Menzies (AAFC-Morden), were used in these trials. The collection locations and dates of the isolates is unknown, but he most recent record of work involving these three isolates is 20 years old (Menzies 2009; Menzies 2001; Menzies and Thomas 1997). An equal mixture of these three isolates was used as inoculum in the University of Saskatchewan field disease nursery (described below). Inoculum used at the University of Minnesota field disease nursery (described below) consisted of a mixture of undefined, local isolates of *U. avenae* collected from naturally infected smutted panicles of the susceptible line PY11108.

3.2.3 Phenotyping

Greenhouse

The DM population and check lines were evaluated for reaction to three different loose smut isolates, A13, A60, and A617. Each isolate was evaluated independently against the population and checks. For each isolate, the DM population was screened twice, and lines that appeared to be resistant were evaluated a third time. For each screening, 30 seeds of each line were planted in three 15 cm (3.78 L) pots (10 seeds/pot) to accommodate the need for a minimum of 15 plants to investigate smut reactions (Menzies et al. 2010). Potting mix was Sunshine Mix #3 comprised of 70-80% Canadian Spaghum peat moss, vermiculite and dolomite limestone (Sun Gro Horticulture Canada Ltd., Seba Beach, AB). The three pots were kept together as one experimental unit to simulate an in-field row. One week after planting 15 mL of Type 100 (14-14-14) Nutricote controlled release fertilizer (Chisso-Asahi Fertilizer Co. Ltd. Tokyo, Japan) was applied to the soil surface. Pots were watered as needed and received Plant-Prod Classic fertilizer (20-20-20, Plant Products Co. Ltd., Brampton, ON) containing approximately 200 mg/L nitrogen once a week. Growing conditions consisted of a 22-23^{o}C daytime temperature, 18-20^{o}C night temperature and an 18-hour day length.

Oat lines were evaluated for reaction to loose smut seven to eight weeks after planting when the panicles were fully emerged from the boot (Zadok's growth stages 51-59). Lines were rated by counting the number of individual plants with one or more smutted panicles and expressed as a percentage of the total number of plants grown for that line. As per current convention, a line with >10% smutted panicles was considered susceptible (Dill-Macky 2014). For each line, the highest infection rate observed was recorded as that its reaction to loose smut.

Field

Inoculated seed of the DM population and check lines were grown in 2013, 2014 and 2015 in the University of Minnesota oat smut field disease nursery located at the Minnesota Agricultural Experiment Station (St. Paul, MN, 44^{o}59'20" N, 93^{o}11'08" W, elevation 296 m). Soil type at the nursery is a Waukegan silt loam (typical Mollisols), with 15-26% clay, 2-4% organic matter and a pH of 5.5-6.5. Oat lines were planted as 1.5 m rows (100 seeds/row), spaced 30 cm apart, in a two replication completely randomized design. Planting occurred between the second and third weeks of April (depending on the year).

In 2015, a field disease nursery, with inoculated DM seed, was also established at the University of Saskatchewan Seed Farm (Saskatoon, SK, 52^{o}08'14" N, 106^{o}36'50" W, elevation 482 m). The field site soil type is an Elstow/Sutherland Association (Chernozem soil) Orthic Dark Brown silty loam with clay texture and a pH range of 6.9 to 8.0 from surface to 61 cm (24") depth. The trial was planted on June 1, 2015, as a two replicate, completely randomized design, with 1.5 m rows (100 seeds/row) spaced 30 cm apart. The trial was irrigated twice.

In all field trials one RIL was not seeded due to an inadequate amount of seed.

Oat lines in all field trials were evaluated for reaction to loose smut approximately seven to eight weeks after planting when the panicles were fully extruded from the boot. Lines were rated using a scale consisting of 0, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, and 90% categories, where individual plant counts were made for the <5% categories and estimates were made for the other categories based on visual

inspection. As per current convention, a line with >10% smutted panicles was considered susceptible (Dill-Macky 2014). For each line the highest infection rate observed was recorded as that line's reaction to loose smut.

3.2.4 Linkage Map Construction, Quantitative Trait Loci Mapping and Statistical Analysis

The DM population was genotyped with the Oat 6K Infinium SNP Assay at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND) on the iSELECT Genotyping BeadChip (Illumina, Inc., San Diego, CA). The genotypic SNP data was screened to remove monomorphic markers, markers with more than 15% missing data, and markers showing skewed segregation (p =0.05). In addition, marker calls were visually examined in GenomeStudio (Illumina, Inc.) and markers with poor clustering were removed. This process produced a set of 737 markers suitable for genetic mapping. Data from these markers were used to create a linkage map in the DM population using JMP Genomics 7 (SAS Institute Inc., Cary, NC). The RI1 cross type (recombinant inbred via selfing) was selected along with the default 0.55 maximum recombination fraction threshold and the False Discovery Rate multiple testing method selected for segregation tests (p-value cut-off set to 0.05). Initial linkage groups were established using the automated hierarchical clustering method with the recombination threshold cut-off set to 0.32. Linkage groups were ordered using the Kosambi map function and the map order optimization algorithm with linkage groups broken if the recombination fraction exceeded 0.32. These parameters were set to effectively balance the number of linkage groups without forcing markers into groups containing large distances between markers.

Output from the linkage mapping procedure was used in conjunction with the phenotypic disease reaction data (input as the highest infection rate observed for each line) for QTL mapping. QTL mapping was conducted in JMP Genomics 7 by simple interval mapping which used the expectation-maximization (EM) QTL mapping model algorithm and tested for the presence of QTL in 2 cM steps. The LOD threshold to declare significance of a QTL was determined for each isolate or field test individually using the permutation test function (set to 10,000 permutations) within MapQTL 5.0 (Kyazma B.V., Wageningen, Netherlands). The LOD thresholds were 3.1 for A60, A617, and the Saskatoon field nursery, and were 3.0 for A13, and the St. Paul, MN field nursery.

Using the 10% smutted panicles as the cut-off to declare a line resistant or susceptible, disease ratings obtained from the individual isolates and field tests were converted to bi-allelic data and the genetic control of resistance was evaluated for each data set using the Yates' Corrected Chi-Square Test (Appendix A). Yates' corrected formula was used in the calculation of the chi-square value because there was only one degree of freedom (Yates 1934). Both one gene (1:1 R:S) and two gene (1:3 R:S) segregation ratios were evaluated, with p = 0.05.

Following the QTL mapping procedure, disease reaction data from a given isolate or field nursery which appeared to be controlled by more than one gene were evaluated for interaction effects between the QTL detected. Overlay plots and output data were used to identify the marker underlying the highest point of each significant QTL peak. Allele data for these markers were obtained for the lines in the DM population and combined with the corresponding phenotypic disease reaction data. Data were analyzed using the Enterprise Guide software portion of SAS 7.1 (SAS Institute Inc.). Disease reaction data were evaluated using Proc GLM using a model in which the fixed effect of each marker and their interaction was evaluated. The assumptions (homogeneous variances and normal distribution of residuals) of analysis of variance (ANOVA) were tested by visual examination of the normal probability plot and residual versus predicted value plot, respectively. Differences between the mean phenotypic values

(disease reactions) associated with each set of allele states was evaluated for significant differences (p = 0.05) using the Bonferroni method.

3.3 Results

3.3.1 Segregation of Loose Smut Reaction

Reaction of the DM $F_{4:7}$ RIL population to loose smut inoculation using three separate isolates (Figs. 3.1, 3.2 and 3.3) in greenhouse trials, and to a mixture of isolates in the Minnesota (Fig. 3.4) and Saskatoon (Fig. 3.5) field disease nurseries, indicated a wide range in infection (Table 3.1 and Appendix B). In all cases 'CDC Dancer' displayed a low level of infection (≤10%), while AC Morgan displayed infection levels exceeding 20% (Table 3.1). Disease incidence of check lines 'Starter' (resistant), 'Ogle' (susceptible), 'Belle' (susceptible), 'Hazel' (susceptible), and PY11108 (susceptible) are reported in Appendix B. The checks displayed expected resistant or susceptible reactions against all single isolates, except isolate A60, and in both field nurseries. For isolate A60 all checks displayed a resistant reaction. Lines from the DM population were designated as having resistant (R) reactions if the greatest smut infection was 10% or less, and were considered to have susceptible (S) reactions when infection was greater than 10%. Based on these classifications, chi-square values were calculated for each trial to test if the R:S ratios fit a one gene model (1:1 R:S) or two gene model (1:3 R:S). Disease segregation data from the DM population, when inoculated with isolate A60, and the complex of A60, A617, and A13 in the Saskatoon disease nursery, fit a 1:1 resistant to susceptible ratio that supports a single gene model for loose smut resistance. A 1:3 resistant to susceptible ratio was in agreement with the disease rating data obtained with isolate A13 and from the mixture of undefined local isolates in the Minnesota disease nursery. Loose smut infection data obtained from isolate A617 did not fit either a one or two gene segregation ratio, but was more similar to the 1:1 ratio, but with an excess of susceptible reactions.

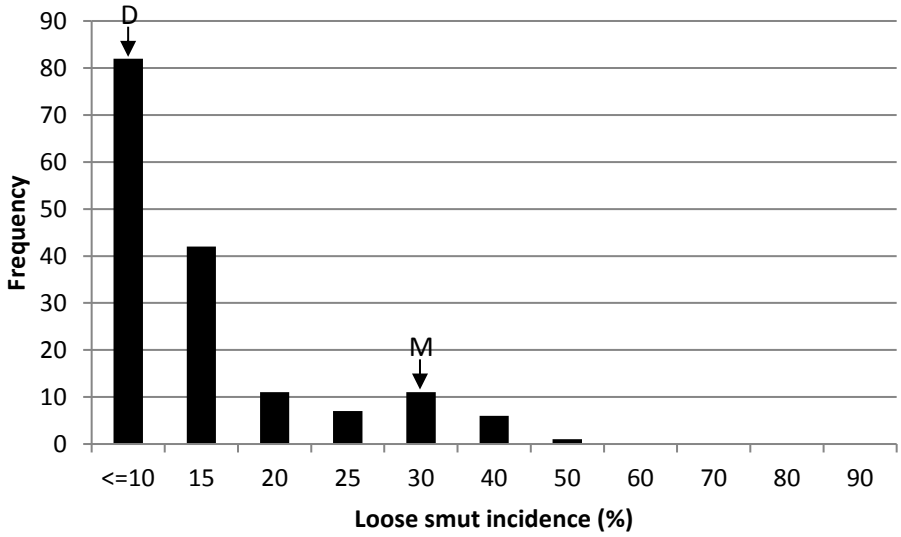

Figure 3.1 Frequency of incidence of the 'CDC Dancer' x 'AC Morgan' oat RIL population to loose smut isolate A60 in the greenhouse trials. D: 'CDC Dancer' incidence; M: 'AC Morgan' incidence.

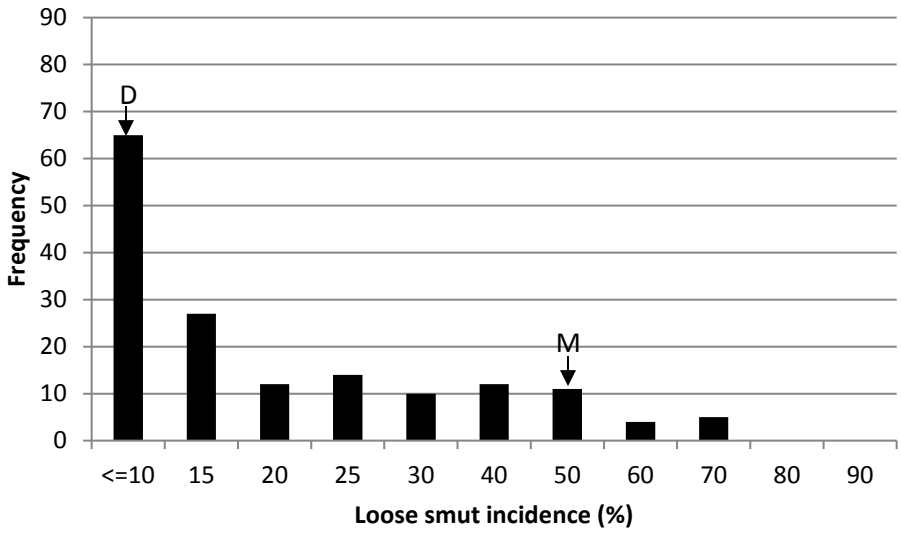

Figure 3.2 Frequency of incidence of the 'CDC Dancer' x 'AC Morgan' oat RIL population to loose smut isolate A617 in the greenhouse trials. D: 'CDC Dancer' incidence; M: 'AC Morgan' incidence.

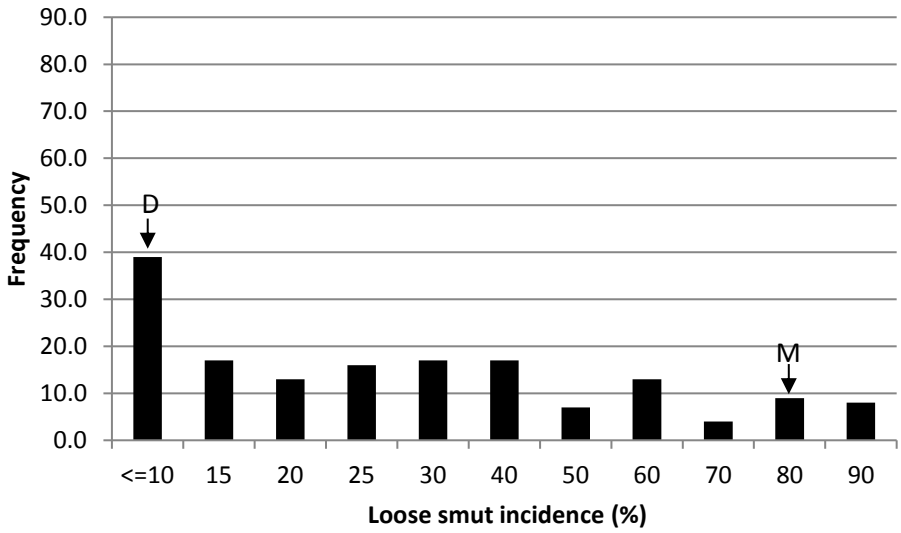

Figure 3.3 Frequency of incidence of the 'CDC Dancer' x 'AC Morgan' oat RIL population to loose smut isolate A13 in the greenhouse trials. D: 'CDC Dancer' incidence; M: 'AC Morgan' incidence.

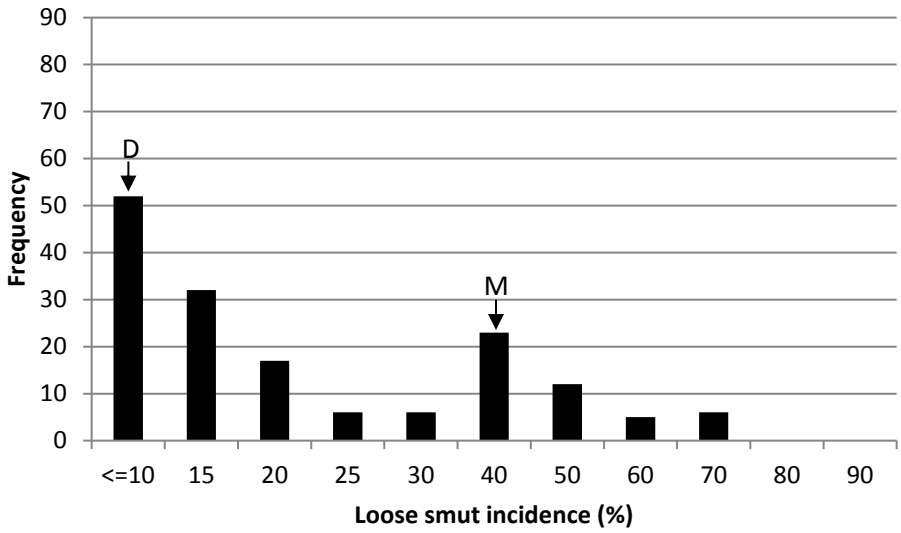

Figure 3.4 Frequency of incidence of the 'CDC Dancer' x 'AC Morgan' oat RIL population to the endemic loose smut isolate mixture in the St. Paul, MN field trials. D: 'CDC Dancer' incidence; M: 'AC Morgan' incidence.

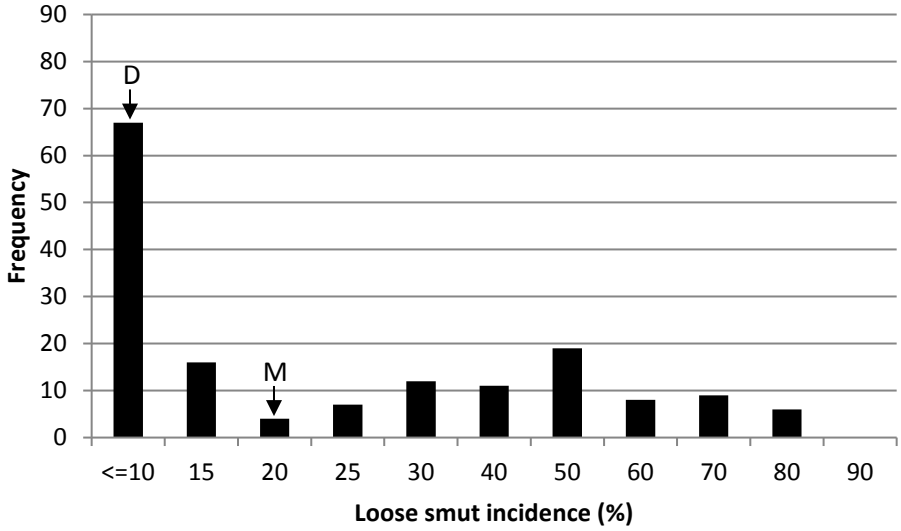

Figure 3.5 Frequency of incidence of the 'CDC Dancer' x 'AC Morgan' oat RIL population to the loose smut isolate mixture (isolates A60, A617, A13) in the 2015 Saskatoon, SK field trial. D: 'CDC Dancer' incidence; M: 'AC Morgan' incidence.

Table 3.1 Evaluation of resistant to susceptible segregation ratios against one and two gene models for loose smut resistance in the 'CDC Dancer' x 'AC Morgan' $F_{4:7}$ RIL population

U. avenae isolate	Nursery	Loose smut reaction			Segregation		ER[a]	X_c^{2b}	*p*- value
		CDC Dancer (R)	AC Morgan (S)	RIL population	R	S			
A60	Greenhouse	0	29	0-47	82	78	1:1	0.05	0.82
							1:3	57.40	<0.001
A617	Greenhouse	0	50	0–70	66	94	1:1	4.55	0.03
							1:3	21.67	<0.001
A13	Greenhouse	4	75	0–89	39	121	1:1	41.06	<0.001
							1:3	0.01	0.92
Mixed[c,d]	MN Field	5	40	1–70	51	108	1:1	19.72	<0.001
							1:3	3.88	0.05
Mixed[c,e]	SK Field	10	20	0-80	68	91	1:1	3.04	0.08
							1:3	25.83	<0.001

[a]Expected ratio

[b]Chi-square test with Yates' continuity correction

[c]One RIL was not grown in the field due to inadequate amount of seed

[d]Mixture of local, undefined isolates collected off the susceptible check, PY11108, which was grown in the Minnesota nursery in the prior year

[e]Mixture of the A13, A60, A617 isolates

3.3.2 Linkage Map Analysis

A total of 34 linkage groups were generated in the DM RIL population using the 737 high quality SNP markers (Table 3.2, Fig. 3.6). All 737 markers were placed on the map, which produced a total map length of 1,094 cM. Marker details can be found in Appendix C. The average distance between markers across the entire genetic map was 2.8 cM; with the largest gap between markers was 35.5 cM (LG24). The largest linkage group was LG23 at 91.5 cM, while LG7 had the greatest number of markers (66). Linkage groups created in the current study encompass portions of all 21 linkage groups present in the oat consensus map presented by Chaffin et al. (2016) (Table 3.2). While many linkage groups aligned to a single consensus map linkage group, LGs 4, 7, 10, 11, 12, 13, 15, 22, 23, 24, 28 and 33 aligned to multiple linkage groups from the consensus map. However, these 12 linkage groups aligned well to individual linkage groups when comparisons were made to the 12 component maps used to build the consensus map (data not shown; see Fig. 3.8 as an example).

Table 3.2 Linkage groups details derived from the 'CDC Dancer' x 'AC Morgan' RIL population Oat 6K SNP data and comparison to oat consensus map linkage groups

DM Linkage Group	Consensus Linkage Group[a]	No. of Markers	Length (cM)	Average Distance (cM)	Largest Gap (cM)
1	3	17	62.6	3.7	20.5
2	2	61	43.6	0.7	13.5
3	23	9	8.9	1.0	4.2
4	20, 17	63	64.6	1.0	27.5
5	19	14	8.4	0.6	2.3
6	24	14	36.9	2.6	20.0
7	1, 28	66	82.0	1.2	22.7
8	17	27	25.6	1.0	4.7
9	20	28	35.0	1.3	11.0
10	15, 9	40	50.9	1.3	18.2
11	33, 21, 17, 2	46	31.2	0.7	10.5
12	18, 1	35	8.6	0.3	6.90
13	18, 4	17	32.3	1.9	15.5
14	5	61	46.9	0.8	13.3
15	9, 6	31	52.0	1.7	25.3
16	19	12	30.1	2.5	19.9
17	13	4	24.4	6.1	24.4
18	11	21	25.5	1.2	16.5
19	6	26	11.5	0.4	4.7
20	3	2	0	0	0
21	8	19	47.2	2.5	18.5
22	9, 8	10	28.9	2.9	22.1
23	9, 18, 21	30	91.5	3.1	27.9
24	12, 9	12	57.1	4.8	35.5
25	1	18	15.7	0.9	7.1
26	23	10	8.7	0.9	6.6
27	15	5	0	0	0
28	1, 9, 3	7	10.4	1.5	6.6
29	13	4	14.7	3.7	12.1
30	12	5	12.9	2.6	9.3
31	8	9	29.5	3.3	12.5
32	24	6	8.9	1.5	3.9
33	13, 1	4	25.2	6.3	11.4
34	33	4	49.7	12.4	24.2

[a]Chaffin et al. (2016)

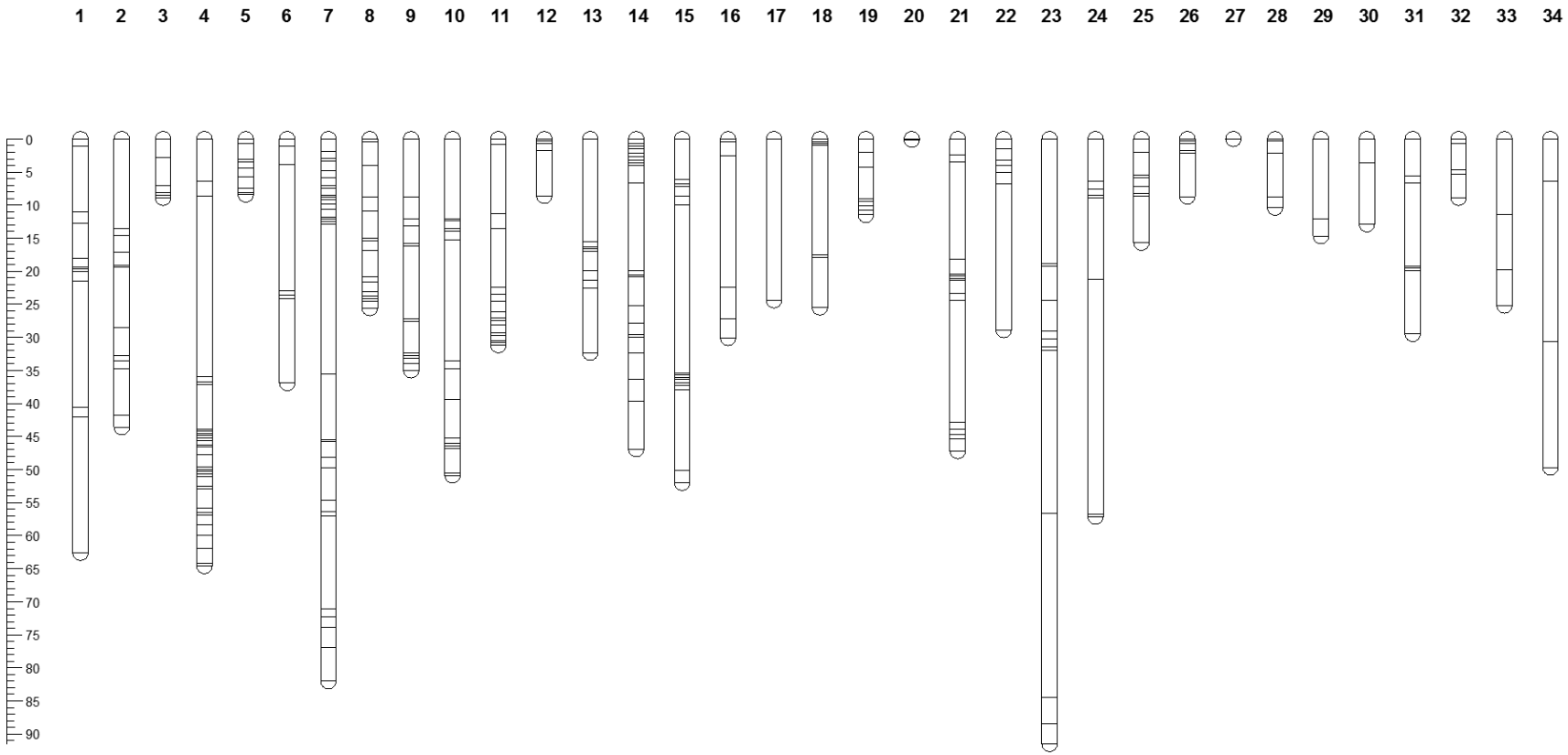

Figure 3.6 A genetic linkage map created using 160 $F_{4:7}$ recombinant inbred lines derived from the oat cross 'CDC Dancer' x 'AC Morgan'. The map indicates the placement of 737 markers distributed across 34 linkage groups. Linkage group number is indicated above each linkage group, horizontal lines within each linkage group indicate the position of a marker and the scale on the left indicates genetic distance in centiMorgans (cM).

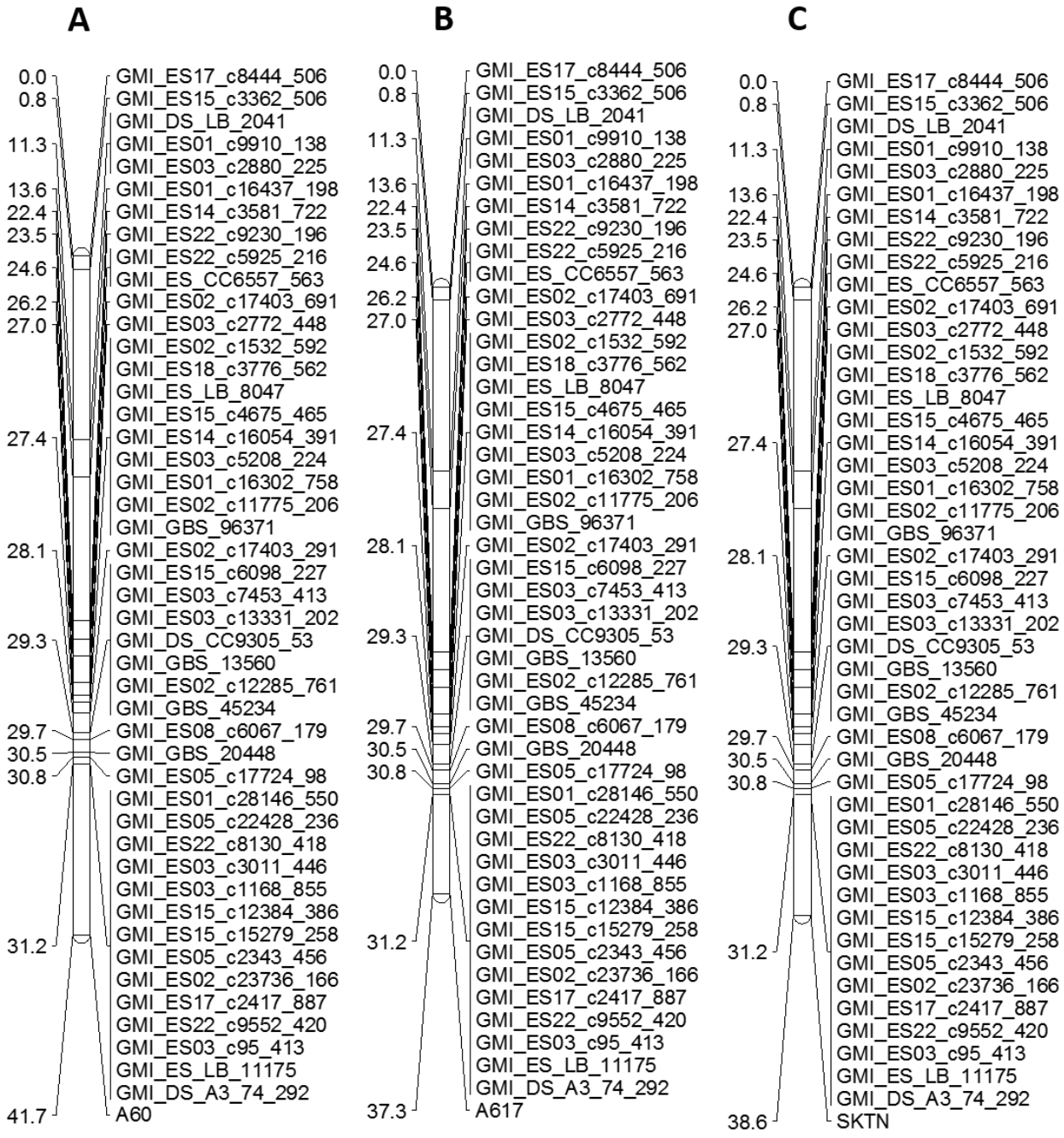

Figure 3.7 Location of the linkage group 11 locus associated with resistance to isolates A60 (A) and A617 (B), and to mixed inoculum (mixture of the A13, A60 and A617 isolates) in the Saskatoon field disease nursery (C). The location of the resistance-associated locus is indicated by the name of the disease screening trial (i.e. A60, A617, or SK). Genetic distance (centiMorgans) is indicated to the left of each linkage group and marker names are indicated to the right of each linkage group.

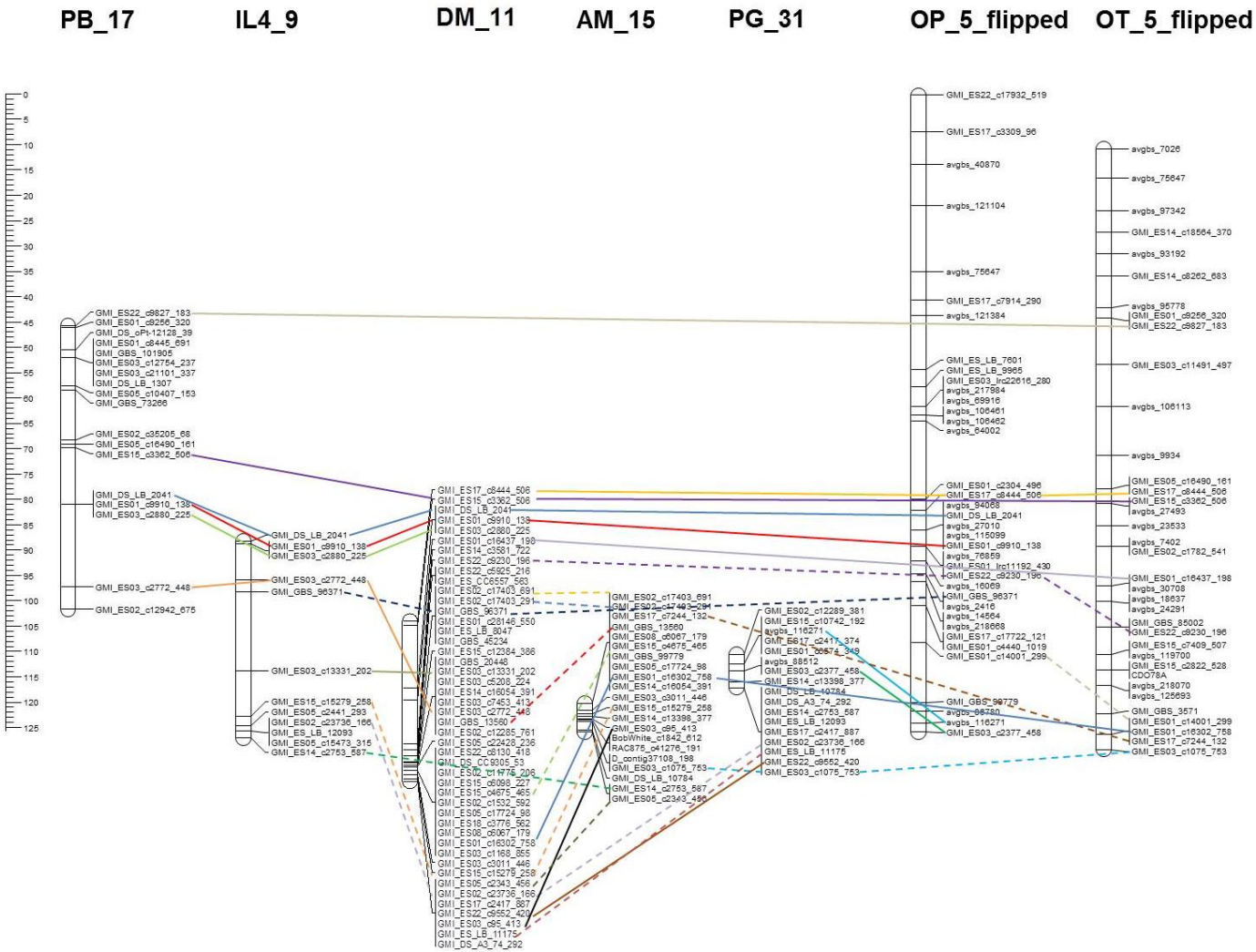

Figure 3.8 Comparison of the 'CDC Dancer' x 'AC Morgan' linkage group 11 (DM_11) with the corresponding linkage groups from six bi-parental component maps used to create the oat consensus map. Coloured lines connect common markers. Population and linkage group number is indicated above each linkage group, marker names are indicated to the right of each linkage and the scale on the left indicates genetic distance in centiMorgans (cM). PB: 'Provena' x 'CDC Boyer', IL4: IL86-1156 x 'Clintland 64', DM: 'CDC Dancer' x 'AC Morgan', AM: 'AC Assiniboia' x MN841801, PG: 'Provena' x 94197A1-9-2-2-5, OP: 'Otana' x PI269616, OT: 'Ogle' x TAMO-301. Flipped indicated the linkage group is in reverse order as indicated in Chaffin et al. (2016).

3.3.3 Genetic Mapping of Loose Smut Resistance

Disease reaction data obtained from the greenhouse screening trials for isolate A60 and the field disease nursery at Saskatoon indicated that a single gene controlled resistance to oat smut. The greenhouse screening trials for isolate A617 also suggested that a single gene was responsible for resistance, although the *p*-value associated with the Chi-Square test fell just within the significance threshold. Despite this, it was treated as a single gene. When these disease reaction data were converted to bi-allelic genotypes (based on the 10% infection cut-off) loose smut resistance mapped to the terminal end of linkage group 11 (LG11) within 6.1-10.5 cM from the nearest marker (GMI_DS_A3_74_292, Fig. 3.7). Differences in the distance can be attributed to variation in designating a line as resistant or susceptible between the different disease screening trials. In most cases this variation arose in lines that were very close to the 10% infection value used to classify lines as resistant or susceptible. Because no flanking marker was identified, LG11 was compared to linkage groups created from the 12 bi-parental component maps used to create the consensus map in the hope of identifying a flanking genetic marker from within the component maps. As indicated in Fig. 3.8, linkage groups from six component maps shared markers present on 'CDC Dancer' x 'AC Morgan' LG11. These linkage groups were derived from 'Provena' x 'CDC Boyer' LG17 (PB, Babiker et al. 2015), IL86-1156 x 'Clintland 64' LG9 (IL4, Foresman 2014), 'AC Assiniboia' x MN841801 LG15 (AM, Lin et al. 2014), 'Provena' x 94197A1-9-2-2-5 LG31 (PG, Oliver et al. 2013), 'Otana' x PI269616 LG5 (OP, Oliver et al. 2013), and 'Ogle' x TAMO-301 LG5 (OT, Kremer et al. 2001). Marker order was consistent across the linkage groups and it was apparent that the loose smut resistance locus resided near the terminus of the component linkage groups. Indeed, the placement of these component maps onto the terminal end of consensus linkage group 33 (Mrg33) also indicated the loose smut resistance locus resided near the terminus of the linkage group.

The disease reaction data obtained from the greenhouse screening trials for isolate A13 and from the field disease nursery in Minnesota indicated that two genes likely controlled resistance to loose smut (Table 3.1). These data were evaluated by QTL analysis to identify loci associated with resistance. In addition, disease reaction data from isolates A60, A617 and the Saskatoon field disease nursery underwent QTL analysis to confirm the location of the resistance locus identified on LG11.

Simple interval QTL mapping based on the loose smut disease reaction ratings (i.e. greatest percent infection rate) for all individual isolates and both field nurseries had a strong QTL peak (LOD scores ranged from 9.2-18.5) at the terminal end of LG11 which was previously associated with resistance to isolates A60 and A617, and in the Saskatoon field disease nursery (Fig. 3.9). In addition, a second, minor QTL on LG19 was also identified for the Minnesota field disease nursery (LOD=4.9). This region also showed a QTL associated with the A13 isolate disease reaction data which was very close to the significance threshold (LOD = 3.0). Comparative mapping of this LG to five of the component maps used in creating the oat consensus map again showed very good conservation of marker order. These LGs were derived from 'CDC Boyer' x 94197A1-9-2-2-5 LG19b (GB, Babiker et al. 2015), AM LG14, OP LG3, 'Kanota' x 'Ogle' LG13 (KO, O'Donoughue et al. 1995) and OT LG12b. In contrast to the LG11 QTL, the terminus of 'CDC Dancer' x 'AC Morgan' LG19 containing the QTL was located in a more central position relative to other component linkages group (i.e. AM14, OP3, KO13 and OT12b) indicating that flanking markers to this QTL should be available (Fig. 3.10). Comparison to these maps also indicated this QTL to be located on consensus LG6.

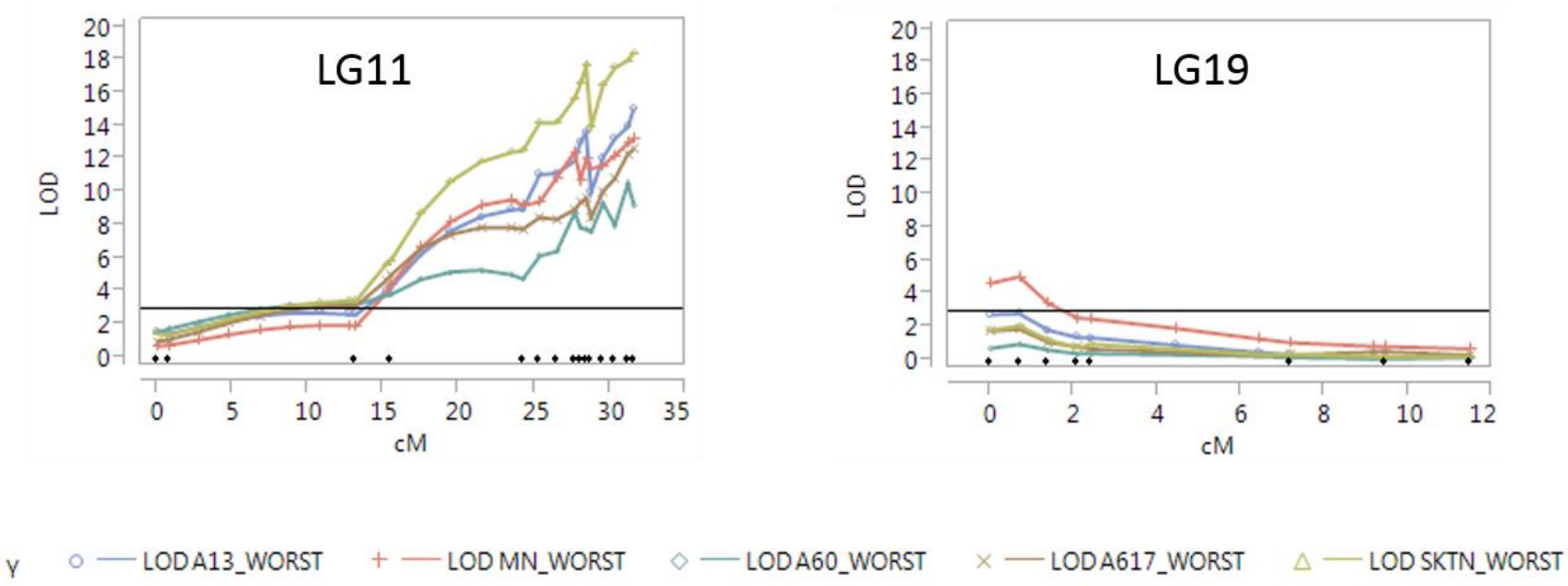

Figure 3.9. Identification of QTL on LG11 and LG19 in the 'CDC Dancer' x 'AC Morgan' RIL population associated with loose smut resistance to isolates A13, A60, A617 in greenhouse trials, mixed inoculum of all three isolates in the Saskatoon field disease nursery, and a mixed inoculum of endemic local isolates from the Minnesota field disease nursery. Genetic distance across each linkage group is indicated in centiMorgans (cM) along the x- axis and LOD score is indicated on the y-axis. The horizontal line in each chart indicates the LOD significance threshold (3.0) and dots along the bottom of each chart indicate the position of markers.

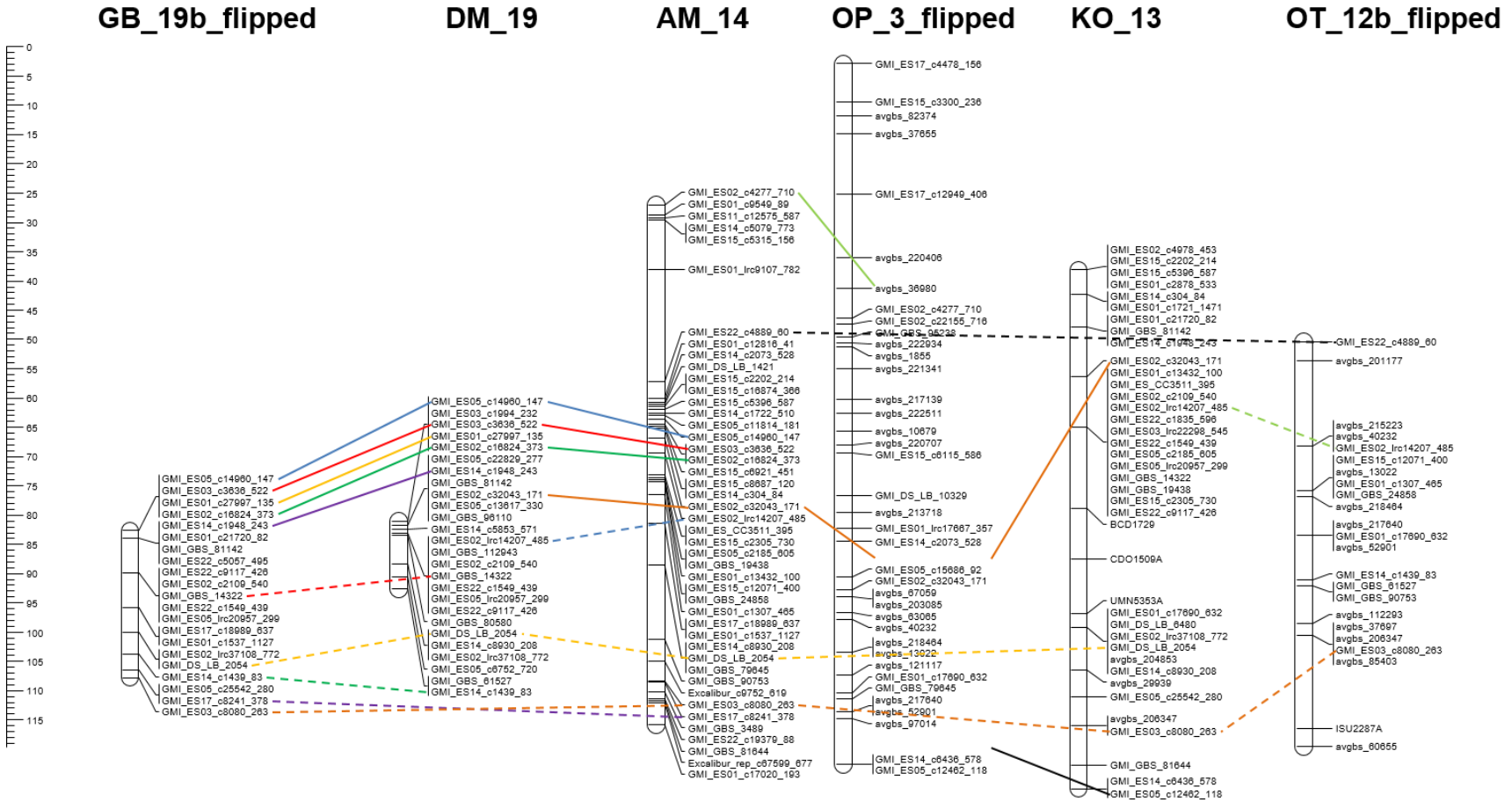

Figure 3.10 Comparison of the 'CDC Dancer' x 'AC Morgan' linkage group 19 (DM_19) with the corresponding linkage groups from five bi-parental component maps used to create the oat consensus map. Coloured lines connect common markers. Population and linkage group number is indicated above each linkage group, marker names are indicated to the right of each linkage and the scale on the left indicates genetic distance in centiMorgans (cM). GB: 'CDC Boyer' x 94197A1-9-2-2-5, DM: 'CDC Dancer' x 'AC Morgan', AM: 'AC Assiniboia' x MN841801, OP: 'Otana' x PI269616, KO: 'Kanota' x 'Ogle', OT: 'Ogle' x TAMO-301. Flipped indicated the linkage group is in reverse order as indicated in Chaffin et al. (2016).

3.3.4 Allele Effects of Loose Smut Resistance Loci

Based on the position of the QTL peaks on LG11 and LG19, SNP markers underlying each peak that contained the fewest missing genotype data points were selected to evaluate the allelic impact on phenotype for each marker, and their interaction in the case of the A13 and MN disease data. The marker GMI_ES15_c15279_258 was selected for the major QTL on LG11, while marker GMI_GBS_96110 was selected for the minor QTL on LG19. For all sets of disease data and both markers, lines carrying the 'CDC Dancer' allele had significantly lower phenotypic disease rating than lines carrying the 'AC Morgan' allele, and these probabilities are summarized in Table 3.3. The average phenotypic effect of a line containing either allele for each marker and their interaction are presented in Figs. 3.11-3.15 for each set of disease reaction data. It was also evident that the presence of the LG11 'CDC Dancer' allele was sufficient to provide resistance (as defined by a 10% cut-off value). Interactions between the two markers with the A13 and MN disease trial data indicated that the presence of either LG19 allele had no impact on phenotype in the presence of the LG11 'CDC Dancer' allele. However, in the absence of the LG11 'CDC Dancer' allele the LG19 'CDC Dancer' allele had a lower phenotypic score than with the LG19 'AC Morgan' allele (Figs. 3.11 and 3.14).

Table 3.3. Probability values (p = 0.05) from the analyses of variances for the fixed effects of the LG11 major QTL, the LG19 minor QTL, and their interaction (for the A13 and MN disease trial data) on loose smut disease reaction in the 'CDC Dancer' x 'AC Morgan' oat population

		Disease Trial				
Marker Effect	LG	A13	A60	A617	MN	SK
GMI_ES15_c15279_258	11	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
GMI_GBS_96110	19	<0.0001	-	-	<0.0001	-
GMI_ES15_c15279_258 x GMI_GBS_96110 QTL	11, 19	0.0051	-	-	0.0010	-

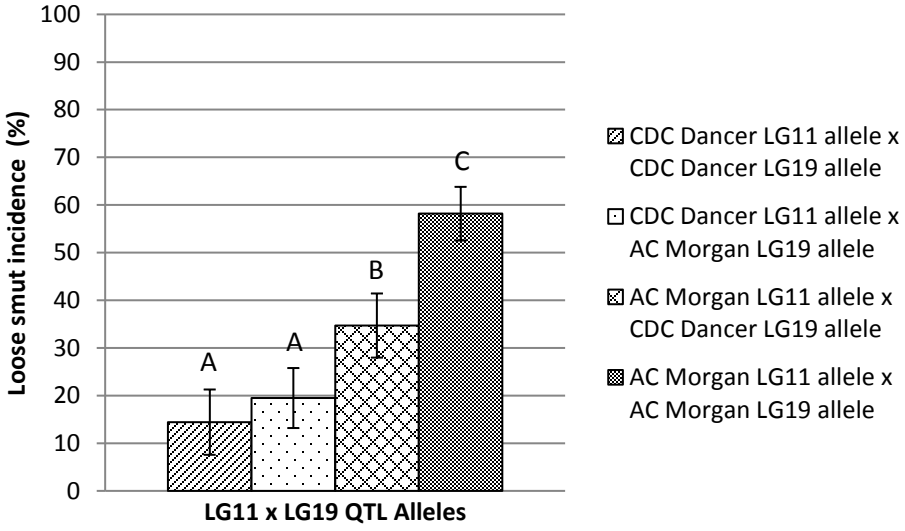

Figure 3.11 Effect of 'CDC Dancer' and 'AC Morgan' alleles at the LG11 major QTL (GMI_ES15_c15279_258) and LG19 minor QTL (GMI_GBS_96110) on the loose smut reaction to *Ustilago avenae* isolate A13 in greenhouse trials.

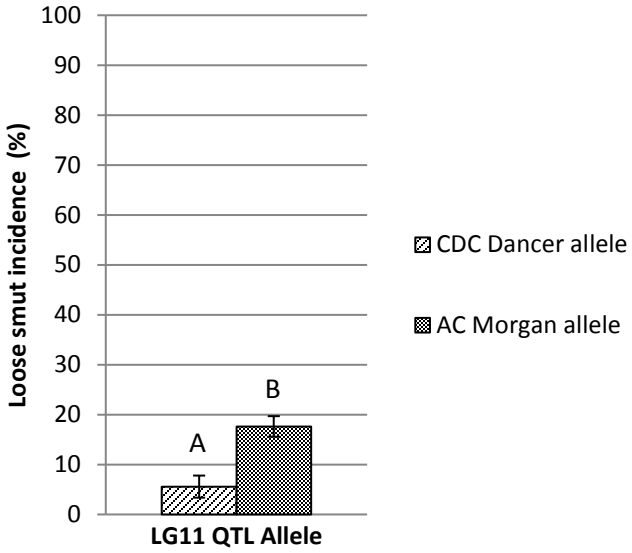

Figure 3.12 Effect of 'CDC Dancer' and 'AC Morgan' alleles at the LG11 major QTL (GMI_ES15_c15279_258) on the loose smut reaction to *Ustilago avenae* isolate A60 in greenhouse trials.

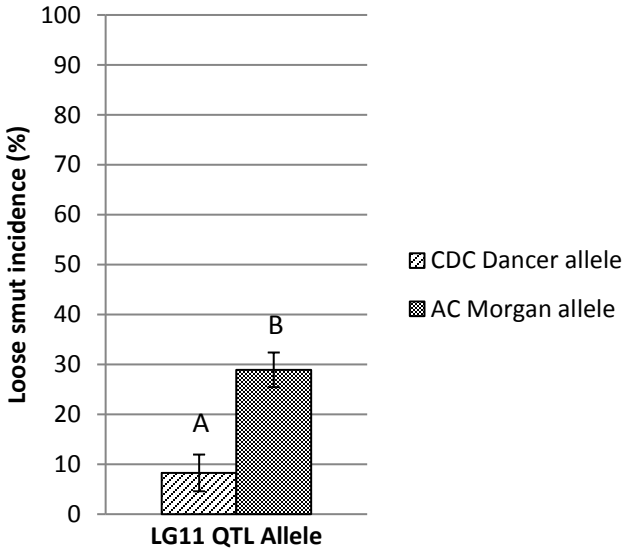

Figure 3.13 Effect of 'CDC Dancer' and 'AC Morgan' alleles at the LG11 major QTL (GMI_ES15_c15279_258) on the loose smut reaction to *Ustilago avenae* isolate A617 in greenhouse trials.

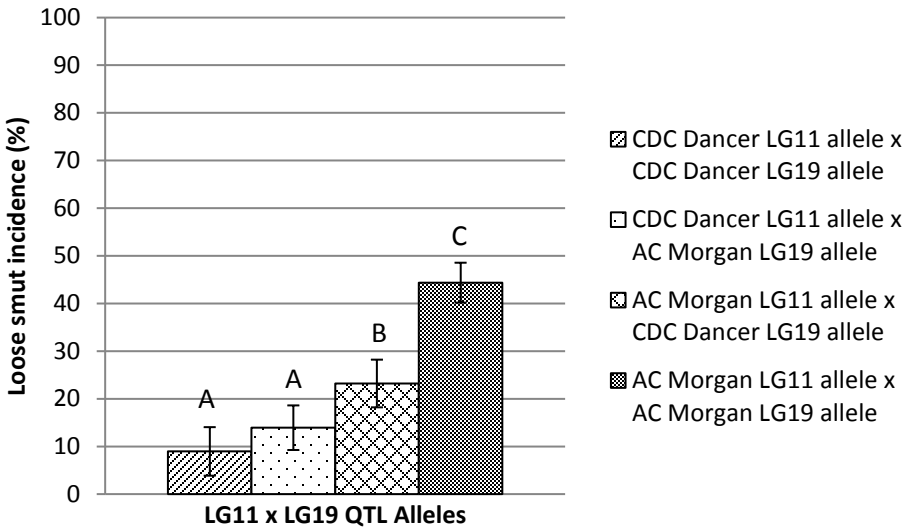

Figure 3.14 Effect of 'CDC Dancer' and 'AC Morgan' alleles at the LG11 major QTL (GMI_ES15_c15279_258) and LG19 minor QTL (GMI_GBS_96110) on the loose smut reaction to endemic *Ustilago avenae* population in St. Paul, MN field trials.

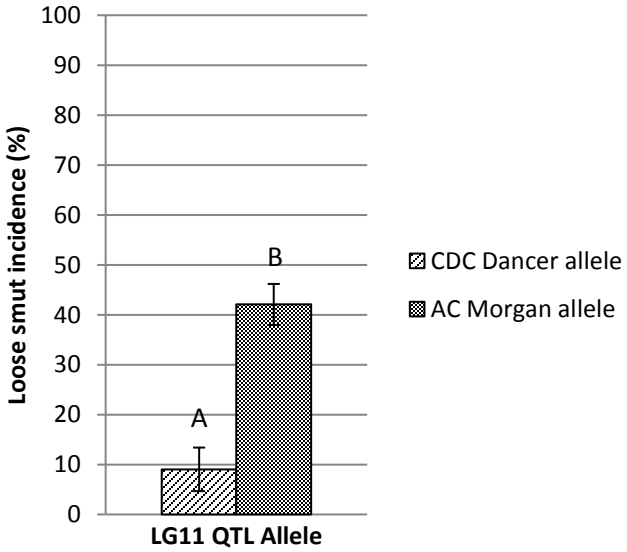

Figure 3.15 Effect of 'CDC Dancer' and 'AC Morgan' alleles at the LG11 major QTL (GMI_ES15_c15279_258) on the loose smut reaction to *Ustilago avenae* isolates A13, A60, A617 in mixture in Saskatoon, SK field trial.

3.4 Discussion

Breeding for oat loose smut resistance is a time- and labour-intensive process. Disease evaluation must wait until full panicle emergence, and because of the possibility of false negatives (disease 'escapes'), lines must be screened several times to ensure the presence of resistance. Therefore, the identification of molecular markers in this study linked to loose smut resistance derived from 'CDC Dancer' will increase the accuracy and efficiency of evaluating this trait within oat breeding programs.

The resistance present in 'CDC Dancer' can be traced back to several potential sources. 'Black Mesdag', 'Burt', and 'Sibiryak' (syn. 'Siberian', OAC 72) have each been determined to carry loose smut resistance over the last century (Appendix D). 'Sibiryak' was considered highly resistant to loose smut; however, the genetic inheritance of its resistance has not been investigated. 'Burt' is a descendant of 'Red Rustproof', which is considered immune to loose smut (Nielsen 1977). Although not in the 'CDC Dancer' pedigree, 'Fulghum' is also derived from 'Red Rustproof'. Its resistance was determined to be expressed in a dominant manner, and this resistance is likely shared with 'Burt' given they both originated from selfing 'Red Rustproof' (Nielsen 1977). 'Black Mesdag' and 'Fulghum' were each determined to carry a single dominant resistance gene, but not the same gene (Reed 1925, 1935). Virulence to 'Black Mesdag' and 'Fulghum' was identified in the early 1990's, with two isolates collected from commercial Canadian oat fields (Menzies and Thomas 1997). Infection rates with the two isolates were observed at 1% and 33% for 'Black Mesdag', and 1% and 12% for 'Fulghum' (Menzies and Thomas 1997). In loose smut collections from the late 1990's, no *U. avenae* isolates virulent on 'Black Mesdag' and' Fulghum' were found (Menzies 2001). Nielsen (1977) identified virulence against both cultivars in the 1970s, but it was not specified where the inoculum had originated. Pathotypes of *U. avenae* collected throughout the 1980s from Minnesota, were virulent on 'Black Mesdag', and pathotypes collected in 1990 were virulent on 'Fulghum' (Wilcoxson and Stuthman 1993). The effectiveness of 'CDC Dancer' resistance within Canada and the University of Minnesota field nursery suggest that resistance is derived from 'Burt' through 'Red Rustproof', or the 'Fulghum' type, given the low infection rate observed by Menzies and Thomas (1997) and the less frequent appearance of pathotypes identified in Minnesota against the 'Fulghum' resistance. However, because the inheritance of 'Sibiryak' resistance is undefined and it has not been used as a check line to evaluate virulence of *U. avenae* isolates under controlled conditions or within field nurseries, it cannot be ruled out as a possible source of 'CDC Dancer' resistance. Based on pedigree analysis it appears that 'CDC Dancer' resistance is not derived from 'Markton', which was used extensively in Western Canadian breeding programs to develop the loose smut resistance cultivars like 'Dumont', 'Robert' and 'AC Preakness' (Menzies and Thomas 1997).

Genetic mapping of loose smut resistance in the DM population consistently identified a major QTL located on LG11 (consensus map linkage group Mrg33) across all single isolate greenhouse trials and in both field disease nurseries. In addition, a minor QTL located on LG19 (consensus map linkage group Mrg6) was detected in response to the Minnesota field disease nursery and the A13 single isolate greenhouse trial. The identification of a lone QTL with the A60 and Saskatoon field nursery data complements the chi-square analysis, which indicated single gene control of resistance. The A617 segregation data was very close to a one gene model of inheritance, which was supported by the identification of a single QTL. The lack of fit for this isolate may have been because a number of lines fell close to the 10% cut-off value used to differentiate resistant from susceptible lines. By using phenotypic values for the QTL analysis, the problem of somewhat arbitrary classification of resistance or susceptibility is avoided and thus the single QTL detected provides a greater measure of confidence in suggesting single gene control of resistance to this isolate. The detection of two QTL governing resistance with the Minnesota field disease nursery and the A13 single isolate greenhouse data are in

agreement with the two gene mode of inheritance determined using the chi-square analysis. Despite the identification of a minor QTL from these two screening trials, assessment of allelic effects across both loci demonstrated that the major gene identified in LG11 was sufficient to provide resistance. The presence or absence of the 'CDC Dancer' allele at the LG11 QTL resulted in significant differences in loose smut reaction with all *U. avenae* isolates and with isolate mixtures used in the field nurseries. The data obtained from the Minnesota field disease nursery and the A13 single isolate greenhouse trial indicated that when the 'CDC Dancer' resistant allele was present at the major LG11 QTL there was no significant difference in disease reaction due to the presence of either allele (i.e. from 'CDC Dancer' or 'AC Morgan') at the minor QTL on LG19. However, the presence of CDC Dancer alleles at both QTL displayed a trend to lower the average disease rating in comparison to when a CDC Dancer allele was present at LG11 and an AC Morgan allele was present at LG19.

Only one other study has attempted to identify and map molecular markers linked to loose smut resistance in oat. Eckstein et al. (2002) evaluated the same three populations in which resistance was derived from 'Markton' and reported that a single locus located on linkage group KO14 in the Kanota x Ogle map (Tinker et al. 2009) governed resistance. The locus was thought to consist of three tightly linked genes, each of which governed resistance to one of the A13, A60 and A617 isolates used in the current study. These finding were in agreement with a previous study, which also concluded that 'Markton' carried three genes for resistance to *U. avenae* (Murphy and Coffman 1961), although prior work by Reed and Stanton (1938) indicated that 'Markton' carried only two resistance genes. Although the KO14 linkage group also aligns to linkage group Mrg6 in the current oat consensus map (Chaffin et al. 2016), the resistance locus mapped by Eckstein et al. (2002) is 40-50 cM from the minor QTL mapped in the current study (data not shown). More significantly, the major QTL mapped in the current study resides on Mrg33 in the consensus map. This definitely indicates the resistance gene in 'CDC Dancer' is not derived from 'Markton', as 'Markton' and its derivatives are not present in the pedigree of 'CDC Dancer'.

'Markton' is still regarded as an effective source of loose smut resistance based on the most recent Canadian surveys As such, mapping the genomic location and developing better molecular markers linked to the gene(s) governing this resistance would be worthwhile (Menzies and Thomas 1997; Menzies 2001; Smith and Bressman 1931). The populations utilized by Eckstein et al. (2002) and Kibite et al. (2004) to map the resistance derived from 'Markton' were very small (33 - 34 RILs) and the closest marker resided at 5-18 cM away. Evaluating a larger population with the Oat 6K Infinium SNP Assay should allow the identification of closer markers. Additionally, by using this chip the genomic location on KO14 can be confirmed or refuted with more confidence. Identifying the genomic location of different loose smut resistance genes, along with linked markers suitable for high-throughput MMAS, will allow testing of existing germplasm and incorporation of both resistance genes into future cultivars.

Simply inherited resistance to oat loose smut has been identified in several other studies as well. Cherewick and McKenzie (1969) determined that the cultivar 'Victoria' possessed one or two dominant resistance genes. Wilcoxson et al. (1993) studied three different crosses and concluded that single gene control of smut resistance (derived from ND820559) was present in one population and two genes were responsible for resistance (derived from MN85320, 'Don' and 'Starter') in the other two populations. Similar inheritance of resistance is also observed with other cereal smut diseases. For example, a single major gene on the short arm of barley chromosome 1H was determined to be responsible for resistance to covered smut, caused by *Ustilago hordei* (Pers.) Lagerh. (Ardiel et al. 2002). Several single resistance genes to barley loose smut, caused by *Ustilago nuda* (Jens.) Rostr. genes have been mapped, including the *Un8* gene on the long arm of chromosomes 1H (Zang et al. 2015) and another gene (possibly *Un6*) on

the long arm of chromosome 3H (Menzies et al. 2010). Two different genes were mapped to the short and long arms of wheat chromosome 5B that governed resistance to loose smut caused by *Ustilago tritici* (Pers.) Rostr. (Kasa et al. 2014; Kasa et al. 2015). In maize, the *ZmWAK* resistance gene underlying a major QTL responsible for resistance to head smut, caused by the soil-borne fungus *Sporisorium reilianum*, was cloned from the long arm of chromosome 2 (Zuo et al. 2015). A single major gene, *Shs*, was also identified that provided resistance in sorghum to the same pathogen, *S. relianum* (Oh et al. 1994; Li et al. 2012). Aitken et al. (2013) concluded that resistance in sugarcane to smut, caused by *S. scitamineum* was associated with a single major gene, but it has yet to be mapped. As work progresses on these diseases it will be interesting to determine if there is a common mechanism of resistance conferred by these genes, if they display similarity in sequence or are found in syntenic regions, suggesting origin from a common ancestral gene.

A total of 34 linkage groups were identified in the DM population, with two (LG11 and LG19) being associated with loose smut resistance. Comparison of these two linkage groups with the component maps used to create the recent oat consensus map (Chaffin et al. 2016) showed very good conservation of marker order. Because the major QTL located on LG11 was found at the end of the linkage group it was hoped that comparison to the component maps might identify flanking makers that could be used during MMAS. However, it was apparent from the linkage groups in each component map that the location of the major QTL is at the end of each linkage group, which corresponded to the distal end of linkage group Mrg33 on the consensus map, likely near the telomere of oat chromosome 15A (according to Chaffin et al. 2016). In the case of the minor QTL located at the end of LG19, it appears from comparison to the component maps that flanking markers can be found for this QTL, located more centrally on linkage group Mrg6, which is associated with oat chromosome 14D (according to Chaffin et al. 2016).

In conclusion, this study identified one major QTL at the telomeric end of oat chromosome 15A that provided a high level of resistance to oat smut across all disease screening trials, while a second minor QTL was found on oat chromosome 14D in some trials. The resistance allele was derived from the parent 'CDC Dancer' and is likely inherited from a resistance source that differs from 'Markton'. Although flanking markers linked to the major QTL will be the focus of future work, the markers identified (GMI_ES15_c15279_258 and GMI_GBS_96110) will be valuable for MMAS of resistance in oat breeding programs.

4.0 Effect of a Loose Smut (*Ustilago avenae*) Resistance Gene on Grain Yield of Oat

4.1 Introduction

One of the primary objectives of plant breeding is to improve yield potential, which is done through a variety of means such as increased grain number, drought tolerance, superior nitrogen use efficiency, enhanced competition with weeds, and better stem or straw strength to name a few (Fehr 1987). Related to improved yield is the concept of yield protection, or the ability of the plant to maximize its genetic yield potential, which is accomplished by incorporating resistance to pests and pathogens.

Disease resistance alleles contribute to yield when disease is present, but as with any allele, there may be associated negative effects (i.e. yield drag). For example, Ortelli et al. (1996) found wheat near isogenic lines (NILs) containing the *Lr9* leaf rust resistance gene had 12% lower yield than the susceptible cultivar used as the recurrent parent. Yield loss was a result of less tillering, a smaller leaf area index (LAI), fewer grains per head, and lower thousand grain weight (Ortelli et al. 1996). Yield reductions were also noted by Singh and Huerta-Espino (1997) with wheat leaf rust resistance. They found that a resistant spring wheat NIL containing *Lr34* had a yield decrease of 5.9% compared to the susceptible NIL (Singh and Huerta-Espino 1997). Kopsich-Obuch et al. (2005) found NILs containing the *rhg1* soybean cyst nematode resistance gene had 132 kg/ha lower yield than the susceptible NILs.

One possible explanation for yield depression is the presence of additional genes that are tightly linked to the resistance gene (i.e. linkage drag). This situation can arise when the source of resistance is a parent that is unadapted, wild or exotic (Concibido et al. 1997; Yuan et al. 2002). For example, the yield drag associated with the *Lr9* leaf rust resistance gene mentioned above was concluded to be the result of linked genes that were introgressed along with the resistance from the unadapted and unrelated *Aegilops umbellulata* (Ortelli et al. 1996). Although molecular markers can greatly minimize this issue, closely-linked genes can still accompany the resistance gene (Brinkman and Frey 1976; Mansur et al. 1996; Concibido et al. 1997). When both parents are adapted, or if the gene has been within a breeding program through numerous cycles of selection, it is less likely that undesirable yield limiting genes will remain linked to the resistance gene since low yield breeding lines will have been selected against in the breeding program.

In other cases, resistance alleles have not been associated with yield reduction, or were actually shown to slightly improve yield over the susceptible line. For example, Brinkman and Frey (1977) recorded increased tillering and more spikelets per panicle in crown rust resistant oat isolines than the recurrent (susceptible) parents. Two experiments involving isoline sets differing for crown rust resistance genes derived from PI 185783 and Wahl 8 identified yield increases of 5.8% and 6.7%, respectively, in field trials (Brinkman and Frey 1977). Similarly, Kabelka et al. (2006) found that resistance to soybean cyst nematode (SCN) in soybean NILs led to agronomic differences, including increased plant height and days to maturity, but reduced lodging, in the susceptible NILs. The resistant NILs, containing either two loci for SCN resistance from *Glycine soja*, or the *rgh1* gene, increased yield by 6% and 7%, respectively, over the susceptible NILs (Kabelka et al. 2006). Transgenic tomatoes that overexpressed the *Pfr* resistance gene, effective against *Pseudomonas syringae* pv. T*omato,* did not result in differences in plant growth or fruit production from the wild-type line (Oldroyd and Staskawicz 1998). Taken together, such studies indicate that predicting the impact of resistance alleles is not possible and must be assessed on a case-by-case basis.

In addition to linkage drag as an explanation for yield depression, some resistance genes have been shown to directly affect yield in the absence of the pathogen by interfering with hormone signaling networks and biochemical processes within the plant that contribute to yield (Denance et al. 2013). This has been referred to as pleiotropy. It is hypothesized that fitness penalties directly associated with resistance genes explain why both resistance and susceptibility alleles are maintained at similar frequencies in populations, that is, if resistance imparted only positive effects to a host, then susceptible alleles would be removed from a population (Tian et al. 2003; Bruns 2016). Although the expression of resistance is commonly considered to cost the plant in terms of diverting resources away from growth, constitutive expression (even at low levels) in the absence of the pathogen is also thought to be costly. For example, Tian et al. (2003) noted that transgenic lines of *A. thaliana* containing a constitutively expressed *RPM1* resistance gene, which provides effective resistance against the bacterial pathogen *Pseudomonas syringae*, produced fewer siliques (pods) and fewer seeds per silique, resulting in a 9% seed reduction, as well as lower shoot biomass than the non-transgenic susceptible line in the absence of the pathogen (Tian et al. 2003).

Phytohormone pathways mediated by ethylene (ET), jasmonic acid (JA) and salicylic acid (SA) have been recognized to play roles in plant defence for some time. More recently it has been recognized that cross-talk occurs between these pathways and those typically associated with growth and development, such as auxin, abscisic acid (ABA), cytokinins (CK), gibberellins (GA) and brassinosteroids (BR). For example, Choi et al. (2010) observed that mutant and transgenic *Arabidopsis* lines that produce high concentrations of CK also showed elevated SA concentrations, which led to enhanced resistance to *Pseudomonas syringae* pv. *tomato.* It was suggested that the interconnectedness of the CK and SA pathways is mediated via shared hormone signalling transcription factors (Choi et al. 2010). In other cases, the interaction between pathways is antagonistic. Salicylic acid has been negatively correlated to production of phytohormones such as auxin, abscisic acid (ABA), gibberellins (GA) and brassinosteroids (BR) (Sun 2011; Kieffer et al. 2010; Gallego-Giraldo et al. 2011). Todesco et al. (2010) found that *Arabidopsis* mutants containing the *Est-1* allele of the *ACD6* (*Accelerated Cell Death 6*) gene had significantly elevated concentrations of SA, but also displayed reduced plant biomass, decreased rates of new leaf development, and necrotic lesions (Todesco et al. 2010). Although seed yield was not evaluated, it can be inferred that slow growth and compromised photosynthetic ability (both by biomass and necrotic lesions) would impact yield. Similarly, Abreu and Munne-Bosch (2009) observed increased growth and large seed yield improvements in *A. thaliana sid2* mutants and *NahG* transgenic lines, both genes which lower SA levels.

Oat loose smut, caused by the pathogen *Ustilago avenae*, causes a direct yield loss in oat when present, by replacing grains with fungal spores. While systemic fungicide seed treatments can control the disease, it is not consistently used due to the cost to the grower. Cultivars with inherent resistance to the pathogen are the most desirable means of disease prevention. Based on the different impacts that resistance genes may have on yield, it is important to understand the implications of incorporating resistance on a case by case basis. Such information will allow breeders to determine the merit of a given resistance gene and decide if the resistance is warranted based on considerations of disease prevalence in a given growing region or alternative control strategies. The purpose of this study was to evaluate the yield impact of oat loose smut resistance present in the cultivar 'CDC Dancer'. This was accomplished by evaluating genetically similar inbred lines, differing for loose smut resistance, in replicated, multi-location yield trials grown under conditions to minimize disease such that the genetic impact of the resistance gene could be isolated. It was hypothesized that there would be no yield effect associated with loose smut resistance.

4.2 Materials and Methods

4.2.1 Recombinant Inbred Lines

An oat population derived from the cross 'CDC Dancer' x 'AC Morgan' (DM) was used as the source of near isogenic lines. The population was comprised of 160 $F_{4:7}$ recombinant inbred lines (RILs) formed by bulking the population until the F_4 generation at which point single panicles where selected to create individual lines which were carried forward to the F_7 generation.

'AC Morgan' was registered in 2000 and was developed from the cross OT526 x OT763 by the Lacombe Research Centre, Agriculture and Agri-Food Canada (Lacombe, Alberta) (Kibite and Menzies 2001). It is a medium maturing cultivar with high yield and desirable grain features including high protein, low oil, low hull percentage, and plump kernels. 'AC Morgan' is susceptible (S) to various diseases, including loose smut of oat. 'CDC Dancer' was derived from the cross OT344 x OT269 and was registered in 2000 by the Crop Development Centre (Saskatoon, Saskatchewan) (Canadian Food Inspection Agency 2017). It is a medium maturing cultivar, with lower yield than 'AC Morgan', but it has excellent grain and milling quality, as well as, resistant (R) to loose smut.

Three pairs of near isogenic lines differing in their reaction to loose smut were identified based on, 1) loose smut disease reaction data obtained from the oat smut field disease nursery located at the University of Minnesota (as described in the previous section), and 2) genotyping data (described below). The near isogenic pairs were T-904-01-151 (R) and T-904-01-163 (S), T-904-01-269 (R) and T-904-01-261 (S), and T-904-01-233 (R) and T-904-01-329 (S).

4.2.2 Genotyping and Near Isogenic Line Identification

The DM population was genotyped with the Oat 6K Infinium SNP Assay at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND) on the iSELECT Genotyping BeadChip (Illumina, Inc., San Diego, CA). SNP data was screened to remove monomorphic markers, markers with more than 10% missing data, and markers showing skewed segregation (p = 0.05). In addition, marker calls were examined visually in GenomeStudio (Illumina, Inc.) and markers with poor clustering were removed. This process produced a set of 610 high quality markers suitable for genetic mapping.

The program NTSYSpc (v. 2.20N) was used to identify near isogenic lines based on a high degree of genetic similarity and to visualize the relationship among lines within the DM population. Marker data (scored as AA = 00, AB = 01, BB = 11, and missing = -999) from the subset of high quality markers was used to produce an association value matrix (SimQual module) based on the Dice coefficient (Dice 1945). Association values were used to cluster the lines (SAHN module) using the unweighted pair group method with the arithmetic mean (UPGMA) method (Sneath and Sokal 1973) to produce a dendrogram (Tree plot module). The validity of the dendrogram was tested by producing a co-phenetic (ultrametric) association value matrix from the dendrogram (Coph module), which was then compared to the original association value matrix (MxComp module). The strength of the relationship between the two matrices is described using the product-moment correlation, r.

4.2.3 Field Trials

The three pairs of near isogenic lines were planted in trials grown in a randomized complete block design with three replications at 12 locations. The locations of the trials were Saskatoon, SK (52°08’25”N, 106°36’50”W, 486m, orthic dark brown, silty loam-clay), Kernen Crop Research Farm, SK (52°09’06”N, 106°31’41”W, 486m, orthic dark brown, clay-clay loam), Goodale Research Farm, SK (52°03’33”N, 106°29’08”W, 486m, orthic dark brown, loam), Kamsack, SK (51°31'30"N, 102°04'42"W, 490m, sandy chernozem), Kelburn, MB (49°47’37”N, 97°14’70”W, 239m, clay loam), Codette, SK (53°16’52”N, 103°52’0.58”W, 372m, grey luvisol), Melfort, SK (52°48’57” N, 104°35’58”W, 469m, black chernozoem), Brandon, MB (49°51'48" N, 99°54'42" W, 490m, thin black), Lacombe, AB (52°27’23”N, 113°44’39”W, 850m, black), Ottawa, ON (45°25’N, 75°41’W, 70m, clay loam), Quebec City, QC (45°36’09”N, 72°33’03”W, 32m, clay loam) and Fargo, ND, USA (n/a). To control for the potential impact on yield caused by disease, seed was treated with Raxil® PRO Shield and plots were sprayed with a half rate of tebuconazole fungicide (Folicur® 250 EW) near the end of tillering and a full rate of azoxystrobin fungicide (Quilt®) once the flag leaf was fully emerged. Field trials were managed according to local practices and details are provided in Appendix E. Grain yield (kg/ha) was collected at each site after drying to a common moisture.

4.2.3 Statistical Analyses

Trial coefficients of variance (CVs) were evaluated and those sites with CVs <15% were used for analysis. The assumptions (normal distribution of residuals and homogeneous variances) of analysis of variance (ANOVA) were tested by visual examination of the normal probability plot and residual versus predicted value plot, respectively. Proc Mixed (SAS v. 9.4, SAS Institute Inc, Cary, NC) was used to determine if lines were significantly different from one another for yield. Line was treated as a fixed effect, while site, block nested within site, and site by line interaction were all considered random effects. As there were significant site and site by line interactions, pairwise comparison of near isogenic lines was done using 95% confidence intervals constructed using Student’s T-test for each location.

4.3 Results

4.3.1 Near Isogenic Line Identification

A total of 4,975 SNPs were interrogated in the DM population using the Oat 6K Infinium Assay. Among these SNPs, 298 were scored as failed or null, 3729 were monomorphic, and 292 had more than 10% missing data. This left a set of 610 high quality SNP markers suitable for genetic analysis that was used in combination with phenotypic disease reaction data from greenhouse and field trials (as described in Chapter 3) to identify near isogenic lines in the population that differed in their reaction to oat smut. One pair of genetically similar lines that showed contrasting reactions to loose smut was identified (Figures 4.1 and 4.2, Table 4.1). Line T-904-01-151 was determined to be resistant to loose smut in all disease screening trials except with the A60 isolate greenhouse trial, while T-904-01-163 was observed to be susceptible in all screening trials. Subsequent disease evaluation of the T-904-01-269 and T-904-01-261 NIL pair and the T-904-01-233 and T-904-01-329 NIL pair showed that all four lines were resistant and were thus not used for further analysis. Of the 610 high quality SNPs these two lines shared 590 similar alleles. The 3729 monomorphic markers shared by these two lines displayed a high percentage of genetic similarity.

Table 4.1 Disease incidence for the near isogenic lines used to study the impact of the loose smut resistance gene derived from 'CDC Dancer' oat on yield

		Loose Smut Reaction (% Infection) of NILs	
***U. avenae* Isolate**	**Nursery**	**T-904-01-151**	**T-904-01-163**
A13	Greenhouse	10	76
A60	Greenhouse	21	29
A617	Greenhouse	5	68
Mixed[a]	MN Field	5	60
Mixed[b]	SK Field	10	50

[a]Mixture of local, undefined isolates collected off the susceptible check, PY11108, which was grown in the Minnesota nursery in the prior year

[b]Mixture of the A13, A60, A617 isolates

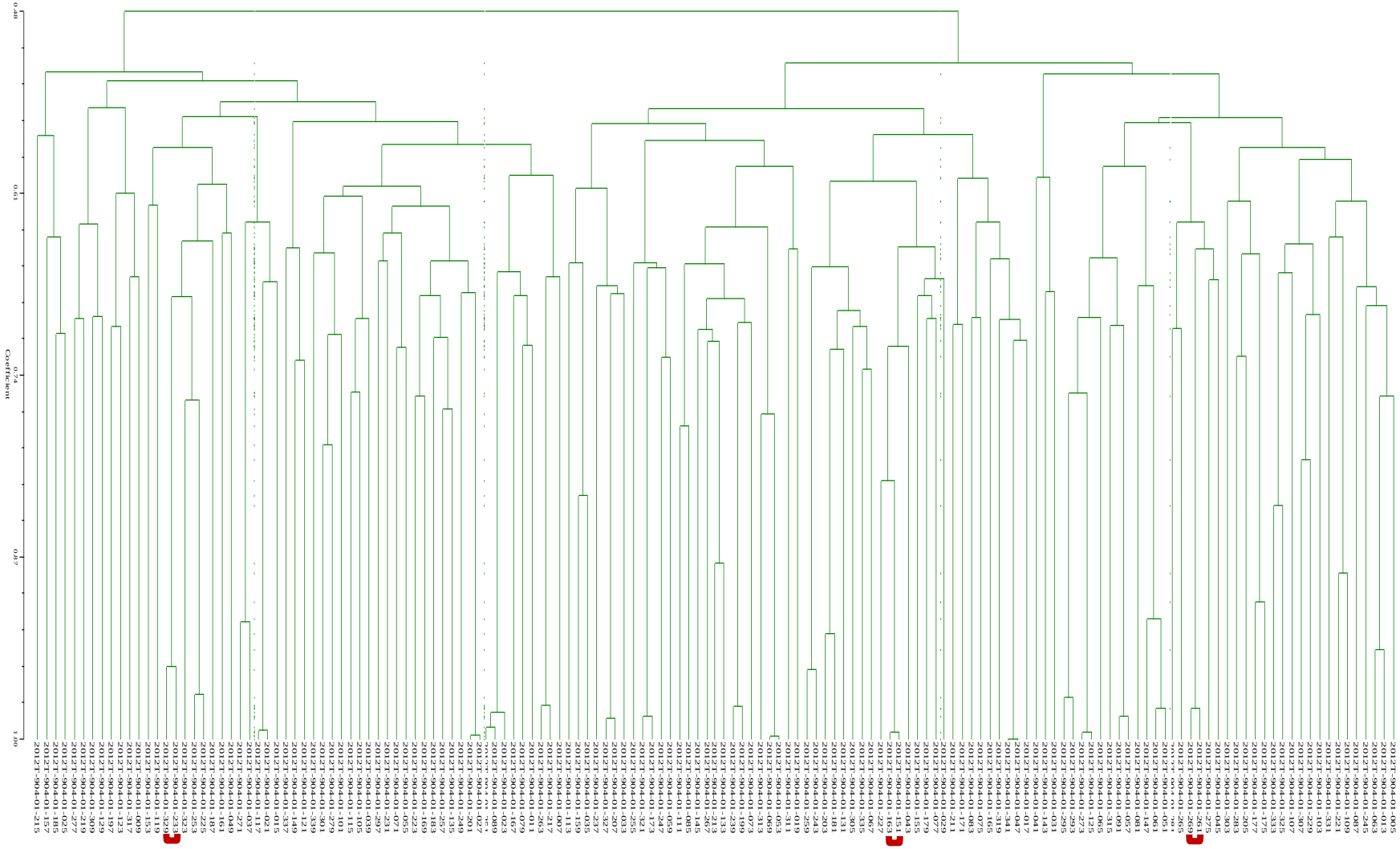

Figure 4.1 Dendrogram generated by UPGMA analysis based on 610 SNP markers showing the genetic relationship among the 'CDC Dancer' x 'AC Morgan' derived $F_{4:7}$ oat population. Red brackets indicate the three pairs of near isogenic lines initially identified to study the impact of the loose smut resistance gene derived from 'CDC Dancer' on yield.

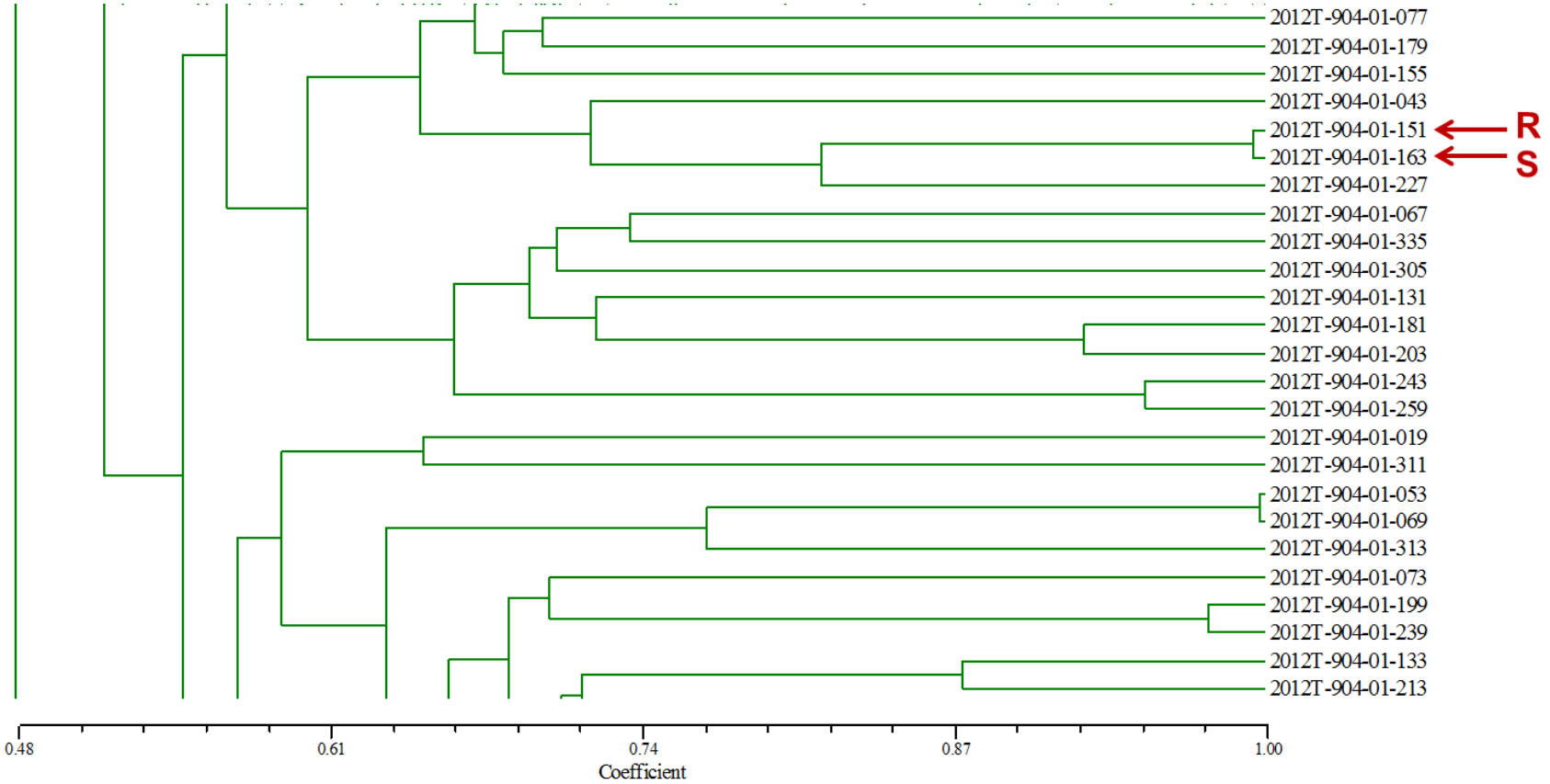

Figure 4.2 Magnified portion of Fig. 4.1 illustrating the genetic similarity of the near isogenic lines, 2012T-904-01-151 and 2012T-904-01-163, from the CDC Dancer' x 'AC Morgan' derived $F_{4:7}$ oat population identified to study the impact of the loose smut resistance gene derived from 'CDC Dancer' on yield. R: resistant line, S: susceptible line. Scale along the bottom of the figure indicates the coefficient of similarity generated by UPGMA analysis based on 610 SNP markers.

4.3.2 Field Trials

Based on the CV criterion, the Codette, SK, Kamsack, SK and Fargo, ND sites were removed. In addition, the Lacombe, AB trial site could not be used due to hail damage to the crop. Full data from the yield trials can be found in Appendix F.

Table 4.2 Coefficients of variation for each of the trial sites used to evaluate the impact of loose smut resistance on yield

Site	Coefficient of Variation (%)
Kernen, SK	9.3
Goodale, SK	11.9
Saskatoon, SK	10.7
Melfort, SK	12.0
Codette, SK	33.5
Kamsack, SK	25.1
Lacombe, AB	-
Kelburn, MB	7.7
Brandon, MB	13.3
Ottawa, ON	10.1
Fargo, ND, USA	18.9
Quebec City, QC	12.2

Data from the remaining eight locations met the assumptions of homogeneity of variance and normal distribution of residuals. Analysis of variance indicated that oat line and blocks within sites were not significant sources of variation, while sites and the oat line x site interaction were (Table 4.3). Sites accounted for more of the variance observed for yield than did the line x site interaction, as determined by variance component estimates. Subsequently, blocks at each site were examined, determined to be non-significant, removed from the analysis and each site was analyzed individually as a completely randomized design to explore the site and oat line by site interaction effects.

Melfort, SK was the only site that demonstrated a significant difference in mean grain yield between the resistant and susceptible line (Table 4.4 and Fig. 4.1).

Table 4.3 Mixed model analysis of variance to assess the impact of variables, including oat line (loose smut resistance), on yield based on data obtained from eight sites in 2015

Source	**DF**	**F value**	**Pr > F**
Oat Line	1	0.69	0.4333
Site	7	10.84	0.0053
Block(site)	16	0.68	0.7728
Oat Line*Site	7	4.55	0.0067
Residual	15		
Total	46		

Table 4.4 Probability values (p = 0.05) from the analyses of variances to assess the impact of oat line (loose smut resistance) on yield at each of the eight sites in 2015.

Site	F value	Pr > F
Brandon, MB	0.36	0.5818
Goodale, SK	1.40	0.3220
Kelburn, SK	0.08	0.7950
Kernen, SK	3.62	0.1298
Melfort, SK	70.52	0.0011
Ottawa, ON	1.86	0.2441
Quebec, QC	5.28	0.0831
Saskatoon, SK	0.47	0.5300

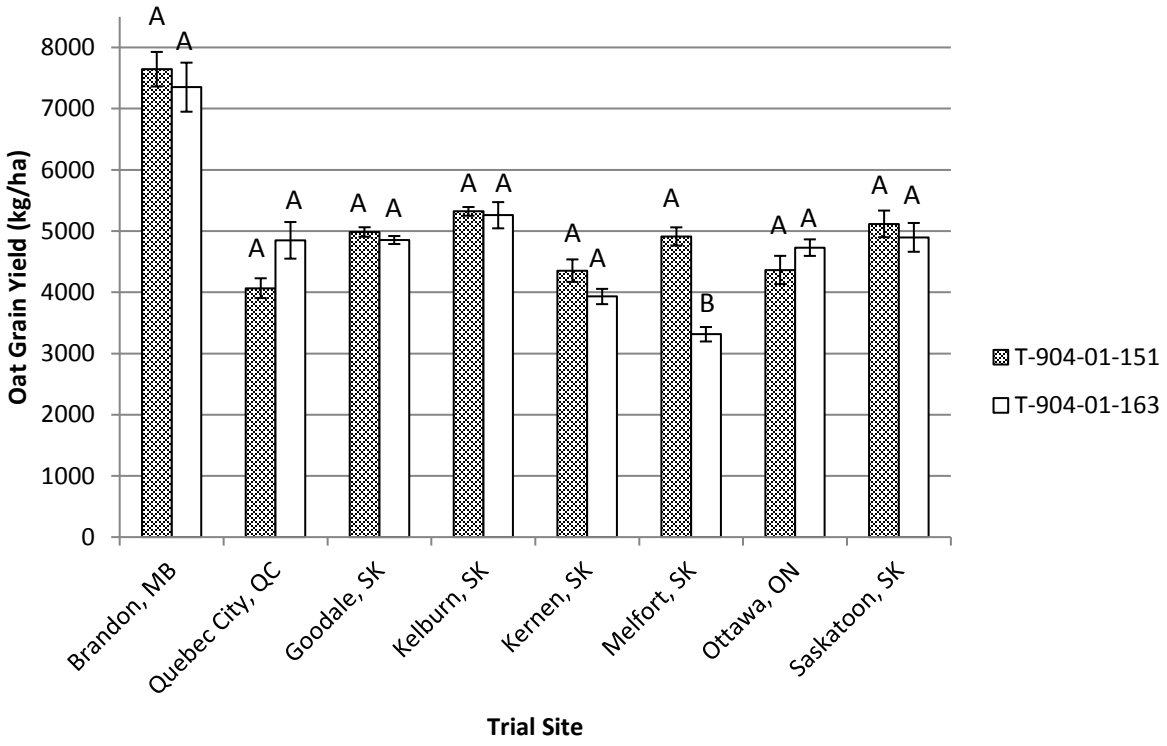

Figure 4.3 Mean grain yield obtained from eight sites in 2015 for the near isogenic lines T-904-01-151 (R) and T-904-01-163 (S) which differ in their reaction to oat loose smut. Bars represent the standard error of the mean for each line at each site. Different letters above the bars within each site indicate significant difference in yield at p < 0.05.

4.4 Discussion

The average grain yield of the near isogenic oat lines T-904-01-151 and T-904-01-163, resistant and susceptible to loose smut respectively, indicated that there was no significant difference when genotype was considered alone. However, a significant genotype by location effect was identified when analyzed in more detail, revealing that most of this effect was due to differences in yield across locations. At only one location (Melfort, SK) was there a significant difference between the two genotypes for yield. This observation may have been due to uneven fertility within the trial, as a row of plots within the trial was observed to be greener and later maturing. In hindsight, this should have been more closely investigated and the site potentially removed, given that the CV test did not eliminate the site based on collected yield variation. Data from further yield trials in 2016 may reveal whether this difference in 2015 was real or not. Based on the complete data set, which represented a diversity of oat growing environments, yield was not negatively impacted by the presence of the smut resistance derived from 'CDC Dancer'.

When investigating the effect of a novel gene or allele, whether for disease resistance or any other trait, it is important to consider the genetic background of the novel allele donor. Many studies that have demonstrated a yield decrease as a result of a resistance gene have used lines derived from crosses where the resistance donor is a non-adapted line. As noted by Ertl et al. (1998), even when using NILs to study the effect of a novel allele, the possibility that yield-limiting genes remain linked to the novel allele is a possibility if additional selection for performance has not occurred. For example, Ortelli et al. (1996) observed a yield penalty in leaf rust resistant winter wheat NILs that derived resistance from the diploid species *Aegilops umbellulata*. It was concluded that despite recurrent backcrossing to the wheat cultivar 'Arina', genes from the unadapted and unrelated *Ae. umbellulata* linked to the resistance gene likely were responsible for the yield penalty (Ortelli et al. 1996). Similarly, two studies involving crown-rust resistant oat isolines detected a yield penalty in resistant lines (Frey and Browning 1971; Brinkman and Frey 1976). Both experiments utilized the same donor parent to develop the NILs, a tetraploid oat derived from an *Avena strigosa* by *A. abyssinica* cross (Frey and Browning 1971; Brinkman and Frey 1976). The current study involved a cross between two elite cultivars and while it is difficult to say with certainty, the resistance gene present in 'CDC Dancer' is likely derived from the AAFC-Winnipeg program via its direct parent OT269 (W90729). The pedigree of OT269 can be traced back to two known sources of smut resistance, 'Black Mesdag' and selections from 'Red Rustproof' such as 'Burt'. In both cases, numerous breeding cycles have occurred between the selection of 'CDC Dancer' and these foundational parents (11 cycles in the case of 'Black Mesdag' and eight in the case of 'Red Rustproof'), which would decrease the likelihood of yield-limiting genes remaining linked to smut resistance. As such, based on the elite nature of the parents used in this study and the absence of any impact on yield (in almost all locations tested), it is fairly certain that the smut resistance gene donated by 'CDC Dancer' is yield neutral.

While linkage drag is often discussed in the context of yield limiting genes linked to novel disease resistance alleles, there are cases where yield increases have been observed. Frey and Browning (1971) and Brinkman and Frey (1976) observed crown rust resistant alleles associated with genes that improved yield. The translocation of the 1RS chromosome arm from rye into hexaploid wheat conferred a yield advantage under no disease pressure (Villareal et al. 1998). These findings emphasize that it is not possible to predict the yield impact of each novel allele that is introduced from exotic germplasm (Frey and Browning 1971).

Although yield penalties associated with resistance genes are often attributed to linkage drag, resistance genes can themselves (even in the absence of a pathogen) interfere, directly or indirectly, with hormone

signalling networks and biochemical pathways in plants, imposing an overall fitness or yield cost by altering the normal functioning of these pathways. Such phenomena are referred to as pleiotropic effects. It is these fitness penalties directly associated with resistance genes that provide an underlying reason as to why resistance and susceptibility alleles are maintained at near-equal frequencies in populations; if resistance attributed only positive effects, the susceptible genotypes would eventually be lost from a population (Tian et al. 2003; Bruns 2016). The implications of resistance genes to plant growth have been reviewed in recent years. Although there is wide variation in the type and amount of fitness penalty associated with resistance genes, the magnitude does not normally exceed 10% (Bruns 2016).

In addition to the well-known roles of ethylene (ET), jasmonic acid (JA) and salicyclic acid (SA) in plant innate immunity, other phytohormones largely involved with plant growth, including auxins, abscisic acid (ABA), cyotkinins (CK), gibberellins and brassinosteroids, have been discovered to play a role in immunity as well. Formerly, they were only thought to contribute to the regulation of growth and development. Interactions between defence hormones and growth hormones can be positive, antagonistic, or both. For example, CK are growth hormones that play a significant role in root and shoot growth, and leaf longevity, but they are also connected to SA signalling (Robert-Seilaniantz et al. 2011). Choi et al. (2010) observed that mutant and transgenic *Arabidopsis* lines, which produce high concentrations of CK, also showed enhanced resistance to *Pseudomonas syringae* pv. *tomato* and elevated SA synthesis. The study suggested that CK and SA may share hormone signalling transcription factors (Choi et al. 2010). Similarly, brassinosteroid concentrations have been demonstrated to be positively correlated with increased SA levels and increased resistance to biotrophic pathogens in *Arabidopsis*, tobacco, and rice (Divi et al. 2010; Nakashita et al. 2003). Salicylic acid is well known to play a role in the induction and regulation of resistance to biotrophic and hemibiotrophic pathogens. Although there does not appear to be an effect of harbouring the loose smut resistance gene derived from 'CDC Dancer' when the pathogen is absent, active resistance to *Ustilago avenae*, a biotrophic pathogen, may influence salicylic acid signalling which could in turn upregulate cytokinin or brassinosteroid biosynthesis. Increased CK or brassinosteroids for the purpose of plant defence would interact with the hormones from roles in root, shoot and leaf development, ultimately impacting yield.

In contrast to CK and brassinosteroids, SA signalling and ABA pathways have been repeatedly noted to be antagonistically correlated (Denance et al. 2013). ABA is a key component in seed development, desiccation, dormancy and abiotic stress, but its involvement with plant defence pathways has also become apparent (Wasilewska et al. 2008). Knockout mutant or ABA-impaired tomato and *Arabidopsis* lines were found to have increased resistance to a broad range of biotrophic and necrotrophic fungal pathogens resulting from over-expression of SA defence pathways (Audenaert et al. 2002; Mohr and Cahill 2003; Garcia-Andrade et al. 2011; Sanchez-Vallet et al. 2012). Similarly, potato plants showed increased susceptibility to biotrophic fungal pathogens when pretreated with ABA, in effect impeding SA signalling (Henfling et al. 1980). *Arabidopsis* mutants overexpressing an ABA biosynthesis enzyme exhibited ABA accumulation and decreased SA levels (Fan et al. 2009). Given its role, a reduction in ABA due to increased SA during a resistance response could decrease plant fitness and yield due to a loss or decrease in seed development regulation or an inability to respond to environmental stresses.

Results from the previous chapter indicated that resistance contributed by 'CDC Dancer' was controlled by a single major gene with a second minor gene also detected in reactions with particular isolates of *U. avenae*. Evaluation of the alleles carried by the resistant RIL T-904-01-151 and the susceptible RIL T-904-01-163 for the two SNP markers residing nearest to the two resistance QTL indicated that both lines carried the same alleles. The same was true for several other SNP markers located slightly farther from

these QTL. This observation provides additional evidence to the conclusion made in the previous chapter that the resistance gene is located distal to the end of the linkage group on which the major QTL resides.

In conclusion, this study determined that the loose smut resistance gene present in 'CDC Dancer' is not associated with any yield penalty. Additional field trials were conducted in 2016 using the two RILs differing in smut reaction to obtain further evidence that the resistance gene is yield neutral. However, based on the current data it can be quite confidently recommended that breeders may incorporate this source of resistance into cultivars without negatively impacting yield when the disease is absent, and in situations in which fungicide seed treatment is not desired, such as with organic growers, it will provide yield protection from this pathogen.

5.0 Overall Discussion and Future Research

Oat is an important cereal crop worldwide, mainly as a food source for humans, but also as livestock feed. As with any crop, oat is affected by a variety of diseases, including loose smut. The first recorded instance of loose smut in Canada was in 1894 (Estey 1994; Agrios 2005). Since then, yield losses from this disease have reached levels as high as 25% in Canada and up to 40% elsewhere (Johnson 1961; Parry 1990; Agrios 2005; Wang 2004). Typically, losses do not exceed 10% due to management of the disease through clean seed, fungicide seed treatments and resistant cultivars. However, the low-input nature of oat production can result in avoidance of seed treatment or certified seed on a regular basis, while the increasing prevalence of organic oat production systems precludes the use of synthetic fungicides. As such, genetic resistance offers a solution to both these issues. Genetic resistance to loose smut has been reported in North American oat cultivars that date back to the 1920's, including 'Black Mesdag', 'Fulghum', Markton' and 'Victoria', with resistance typically governed by one or two dominant genes (Cherewich and McKenzie 1969, Reed 1925, Reed 1935, Reed and Stanton 1938).

The first study of this thesis explored the genetic control and genomic location of one source of resistance to *Ustilago avenae* derived from the cultivar 'CDC Dancer'. This study determined that a single major QTL conferred resistance and was effective against isolates present in Western Canada and Minnesota (Chapter 3). This was in agreement with the two hypotheses, that is, that resistance would be governed by a single gene, generated prior to the study. The QTL was located to the terminus of LG11, which corresponds to the telomeric end of chromosome 15A. Unfortunately, markers were identified on only one flank of the QTL. An important goal of future work would be to identify a set of markers on the other end of this QTL. In fact, this work is currently being undertaken within the Crop Development Centre. Even without flanking markers, the current set of markers identified in this study will be useful for MMAS and should help improve the efficiency of breeding for loose smut resistance. Traditionally, evaluation of loose smut reaction is a time- and labour-intensive process since phenotyping must wait until full panicle emergence and because of false negatives (disease 'escapes'), which necessitates that oat lines be screened several times to ensure the presence of resistance.

The placement of 'CDC Dancer' resistance on oat chromosome 15A provides a starting point against which future loose smut resistance genes may be compared. For example, 'Markton' is still regarded as an effective source of loose smut resistance and therefore mapping the genomic location and developing better molecular markers linked to the gene(s) governing this resistance would be a worthwhile effort of future studies (Menzies and Thomas 1997; Menzies 2001; Smith and Bressman 1931). Comparison of the mapping work done on the 'Markton' resistance by Eckstein et al. (2002) and Kibite et al. (2004) suggest it is present at a different location from the 'CDC Dancer' resistance within the oat genome. Identifying markers linked to the 'Markton' resistance that are suitable for high-throughput MMAS will allow incorporation of both resistance genes into future cultivars. However, it may also be prudent to maintain the resistances in separate cultivars to allow growers to alternate sources of genetic resistance to oat loose smut and mitigate the potential to develop *U. avenae* populations virulent against both genes. However, this would take a greater level of coordination among breeders and growers than is likely feasible.

Surveys evaluating the virulence of *Ustilago avenae* populations in Western Canada have been abandoned, but the disease is still evaluated in oat breeding programs. There is no record of the original date of collection of the isolates used in this study, but they are known to be about 20 years old (Menzies, personal communication). Given the rate at which virulence appeared in the past, it would be practical to re-initiate surveys and assess virulence of isolates collected, against a common set of

differentials, perhaps every ten years, to provide an occasional picture of the virulence profile across the prairies (Reed and Stanton 1942) and to ensure that breeding efforts are focussed on relevant virulence.

The second study of this thesis explored the question of whether possessing loose smut resistance (derived from 'CDC Dancer') was associated with a yield difference when the pathogen was absent. The results of this work indicated that incorporating this resistance gene should be pursued as no yield effect was evident using near isogenic lines differing in their loose smut reaction (Chapter 4). This confirmed the hypothesis that no yield penalty would be identified with the true loose smut resistance gene from 'CDC Dancer'. Given the long breeding history of using 'CDC Dancer' resistance, it is evident that any negative genes linked to the resistance have been removed or never existed. Linkage drag, as observed by Ortelli et al. (1996) when working with wheat NILs differing for the presence of the *Lr9* leaf rust resistance gene, is one explanation for yield penalties associated with resistance genes, often derived from wild germplasm. Alternatively, yield penalties due to the resistance gene itself, such as those observed with the *mlo* gene (Schwarzbach 1976; Jorgensen 1992; Smedegaard-Petersen and Stolen 1981) or the *A. thaliana RPM1* resistance gene (Tian et al. 2003), are thought to result from disruption of normal hormone regulation in the plant that causes plant resources to be utilized in defensive mechanisms, as opposed to growth, when pathogens are not present (Denance et al. 2013). Such negative associations with the loose smut resistance derived from 'CDC Dancer' were not evident.

This study was based on data from one year (2015) of yield trials in the field. While this provided evidence that no significant yield impact existed, additional yield trials at multiple locations would provide increased support for the findings. In fact, a second year of yield trials was completed in 2016 to supply this information.

Additional studies to investigate the interaction of the pathogen and host may be beneficial. Microscopy coupled with PCR to detect pathogen DNA at different growth stages and tissues within the oat plant could illuminate when and where the resistance mechanism occurs. The molecular markers identified in this thesis will be helpful for cloning this gene at such time in the future when genetic tools, such as bacterial artificial chromosome (BAC) libraries and a reference genome sequence become available in oat. Sequencing of candidate genes and employing BLAST searches might facilitate and understanding of gene function and may identify homology to smut resistance genes in other grass species, such as the maize head smut resistance gene *ZmWAK* (Zuo et al. 2015). Understanding the phytohormone and plant defence pathways through which this resistance gene acts may be particularly interesting given the lack of yield penalty associated with the resistance.

In conclusion, this study contributed pertinent information on the existence and location of genetic resistance to *U. avenae* derived from 'CDC Dancer'. Molecular markers linked to this gene, which were identified in this study, could be used to enhance breeding for loose smut resistance through the use of MMAS, increasing the efficiency with which resistant lines can be distinguished and incorporated into agronomically desirable lines. Additionally, this resistance could be used without concern for an associated yield penalty as none was identified with the resistance gene. These findings can help maintain loose smut as a minor disease by mitigating the potential for the evolution of fungicide tolerant *U. avenae* populations through the use of plant resistance and providing advantages to growers under all growing systems.

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7.0 Appendices

Appendix A
Yate's Chi-Square Formula

$$\chi^2 = \sum \frac{[|O - E| - 0.5]^2}{E}$$

Appendix B
Loose Smut Disease Reaction Ratings for the CDC Dancer x AC Morgan Population

Table B1. Loose smut disease reaction ratings for the 'CDC Dancer' x 'AC Morgan' population in greenhouse and field trials in Minnesota and Saskatchewan.

Line	A13-1	A13-2	A13-3	A13 highest	A60-1	A60-2	A60-3	A60 highest
T-904-01-005	11.1	0.0		11.1	3.6	0.0	0.0	3.6
T-904-01-007	75.9	6.9		75.9	8.3	3.7		8.3
T-904-01-009	8.3	0.0		8.3	0.0	0.0	0.0	0.0
T-904-01-011	81.5	14.8		81.5	25.0	15.4		25.0
T-904-01-013	31.0	17.9		31.0	3.7	0.0	31.8	31.8
T-904-01-015	51.7	10.3		51.7	28.6	20.7		28.6
T-904-01-017	86.2	20.0		86.2	3.6	7.4		7.4
T-904-01-019	0.0	7.1		7.1	0.0	0.0	12.5	12.5
T-904-01-021	76.7	7.4		76.7	11.5	30.8		30.8
T-904-01-023	21.4	7.4		21.4	11.1	15.4		15.4
T-904-01-025	6.9	0.0		6.9	4.3	0.0	10.0	10.0
T-904-01-027	7.4	0.0		7.4	0.0	0.0	3.6	3.6
T-904-01-029	8.7	6.9		8.7	0.0	0.0	0.0	0.0
T-904-01-031	23.1	0.0		23.1	0.0	3.7	11.5	11.5
T-904-01-033	42.9	7.1		25.0	0.0	0.0	44.4	44.4
T-904-01-035	32.1	3.3		32.1	0.0	7.4		7.4
T-904-01-037	14.3	0.0		14.3	3.8	0.0	3.4	3.8
T-904-01-039	17.9	3.3		17.9	0.0	0.0	11.1	11.1
T-904-01-041	63.0	7.1		63.0	7.4	4.8		7.4
T-904-01-043	32.1	6.9		32.1	11.1	19.2		19.2
T-904-01-045	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T-904-01-047	23.1	7.4		23.1	7.7	0.0		7.7
T-904-01-049	26.7	0.0		26.7	15.4	0.0		15.4
T-904-01-051	80.0	7.7		80.0	14.3	36.0		36.0
T-904-01-053	43.3	18.5		43.3	37.9	46.7		46.7
T-904-01-055	57.7	36.7		57.7	10.7	13.8		13.8

Line	A13-1	A13-2	A13-3	A13 highest	A60-1	A60-2	A60-3	A60 highest
T-904-01-057	51.9	21.4		51.9	6.9	3.3		6.9
T-904-01-059	29.2	4.0		29.2	6.9	3.4		6.9
T-904-01-061	85.7	41.4		85.7	44.0	41.4		44.0
T-904-01-063	51.9	6.9		51.9	3.4	0.0	30.4	30.4
T-904-01-065	55.6	46.4		55.6	25.0	17.9		25.0
T-904-01-067	6.7	7.4		7.4	4.0	7.7		7.7
T-904-01-069	85.2	24.1		85.2	35.7	28.6		35.7
T-904-01-071	17.4	3.4		17.4	4.0	0.0	8.3	8.3
T-904-01-073	10.0	6.9		10.0	0.0	3.7	0.0	3.7
T-904-01-075	7.7	10.3		10.3	0.0	3.7	7.1	7.1
T-904-01-077	21.4	0.0		21.4	0.0	3.4	8.0	8.0
T-904-01-079	53.8	21.4		53.8	14.3	13.8		14.3
T-904-01-081	72.4	30.0		72.4	20.7	34.5		34.5
T-904-01-083	86.2	11.5		86.2	40.0	21.7		40.0
T-904-01-085	21.4	7.7		21.4	11.5	0.0		11.5
T-904-01-087	71.4	12.0		71.4	19.2	24.1		24.1
T-904-01-089	69.2	18.5		69.2	11.5	7.1		17.1
T-904-01-091	86.2	26.7		86.2	6.7	13.8		13.8
T-904-01-101	23.3	7.1		23.3	0.0	10.0		10.0
T-904-01-103	6.9	13.3		13.3	0.0	0.0	7.4	7.4
T-904-01-105	69.0	26.7		69.0	18.2	7.4		18.2
T-904-01-107	57.7	8.0		57.7	21.4	4.3		21.4
T-904-01-109	40.7	7.1		40.7	3.8	0.0	7.4	7.4
T-904-01-111	34.5	11.1		34.5	3.6	0.0	25.9	25.9
T-904-01-113	30.8	10.7		30.8	0.0	0.0	28.6	28.6
T-904-01-115	42.3	3.4		42.3	3.6	17.4		17.4
T-904-01-117	48.1	3.7		48.1	19.2	25.0		25.0
T-904-01-119	26.7	3.7		26.7	0.0	3.4	3.8	3.8
T-904-01-121	0.0	3.8	11.5	11.5	0.0	0.0	13.8	13.8
T-904-01-123	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T-904-01-125	89.3	44.8		89.3	22.2	17.2		22.2

Line	A13-1	A13-2	A13-3	A13 highest	A60-1	A60-2	A60-3	A60 highest
T-904-01-129	62.5	25.0		62.5	22.2	16.0		22.2
T-904-01-131	9.5	6.9		9.5	0.0	0.0	15.4	15.4
T-904-01-133	10.0	7.1		10.0	7.1	3.8		7.1
T-904-01-135	26.9	10.3		26.9	10.0	10.7		10.7
T-904-01-137	0.0	0.0	27.8	27.8	0.0	0.0	0.0	0.0
T-904-01-139	3.6	0.0	32.9	32.9	0.0	0.0	0.0	0.0
T-904-01-141	6.9	7.1		7.1	3.6	3.4	14.3	14.3
T-904-01-143	34.5	10.3		34.5	11.1	6.9		11.1
T-904-01-145	62.1	22.2		62.1	21.7	13.0		21.7
T-904-01-147	16.7	0.0		16.7	0.0	0.0	4.2	4.2
T-904-01-149	6.9	3.6		6.9	0.0	0.0	8.0	8.0
T-904-01-151	10.7	0.0		10.7	0.0	0.0	21.4	21.4
T-904-01-153	58.6	0.0		58.6	30.8	16.7		30.8
T-904-01-155	86.2	7.7		86.2	15.4	6.9		15.4
T-904-01-157	40.0	0.0		40.0	17.2	10.3		17.2
T-904-01-159	22.2	8.0		22.2	0.0	0.0	0.0	0.0
T-904-01-161	7.7	3.6		7.7	4.0	0.0	4.8	4.8
T-904-01-163	76.7	20.0		76.7	28.6	7.1		28.6
T-904-01-165	56.7	26.9		56.7	7.4	17.2		17.2
T-904-01-167	10.0	14.8		14.8	3.7	7.1		7.1
T-904-01-169	32.0	3.7		32.0	12.0	8.0		12.0
T-904-01-171	16.0	3.3		16.0	0.0	0.0	0.0	0.0
T-904-01-175	37.0	0.0		37.0	3.6	8.3		8.3
T-904-01-177	35.7	0.0		35.7	10.0	12.5		12.5
T-904-01-179	11.5	4.0		11.5	0.0	0.0	3.8	3.8
T-904-01-181	11.5	0.0		11.5	10.7	0.0		10.7
T-904-01-183	53.3	42.9		53.3	31.0	43.3		43.3
T-904-01-185	14.3	3.3		14.3	3.4	0.0	4.8	4.8
T-904-01-187	4.5	0.0	25.5	25.5	0.0	0.0	3.6	3.6
T-904-01-197	26.9	3.8		26.9	4.0	0.0	7.4	7.4
T-904-01-199	17.9	0.0		17.9	0.0	0.0	7.1	7.1

Line	A13-1	A13-2	A13-3	A13 highest	A60-1	A60-2	A60-3	A60 highest
T-904-01-201	16.7	3.4		16.7	0.0	0.0	4.0	4.0
T-904-01-203	0.0	0.0	6.9	6.9	0.0	0.0	3.4	3.4
T-904-01-205	6.9	0.0		6.9	0.0	0.0	0.0	0.0
T-904-01-207	28.6	14.8		28.6	4.0	3.8	13.8	13.8
T-904-01-211	7.4	0.0		7.4	8.0	0.0		8.0
T-904-01-213	40.0	30.0		40.0	3.6	7.4		7.4
T-904-01-215	21.4	0.0		21.4	0.0	0.0	3.6	3.6
T-904-01-217	17.4	12.0		17.4	0.0	3.4	11.5	11.5
T-904-01-219	42.9	18.5		42.9	33.3	7.4		33.3
T-904-01-221	42.3	14.8		42.3	14.8	0.0		14.8
T-904-01-223	56.7	7.1		56.7	0.0	6.9		6.9
T-904-01-225	0.0	0.0	0.0	0.0	0.0	0.0	3.6	3.6
T-904-01-227	4.2	0.0	11.9	11.9	0.0	0.0	4.0	4.0
T-904-01-229	19.2	3.7		19.2	0.0	3.4	25.9	25.9
T-904-01-231	40.0	7.1		40.0	11.1	3.8		11.1
T-904-01-233	4.3	0.0	12.6	12.6	0.0	0.0	0.0	0.0
T-904-01-235	8.3	0.0		8.3	0.0	0.0	4.2	4.2
T-904-01-237	25.9	0.0		25.9	0.0	3.4	14.8	14.8
T-904-01-239	10.3	0.0		10.3	0.0	3.8	3.7	3.8
T-904-01-241	30.8	3.8		30.8	3.7	4.0	25.0	25.0
T-904-01-243	63.3	7.1		63.3	0.0	6.9		6.9
T-904-01-245	42.3	3.4		42.3	0.0	8.0		8.0
T-904-01-247	43.3	10.3		43.3	0.0	7.7		7.7
T-904-01-251	42.9	17.9		42.9	6.9	18.5		18.5
T-904-01-253	11.5	0.0		11.5	0.0	8.3		8.3
T-904-01-255	17.2	3.7		17.2	6.9	14.8		14.8
T-904-01-257	16.7	0.0		16.7	3.8	0.0	3.3	3.8
T-904-01-259	26.7	6.7		26.7	3.8	3.7	11.1	11.1
T-904-01-261	23.1	4.3		23.1	0.0	0.0	0.0	0.0
T-904-01-263	30.8	6.9		30.8	12.0	0.0		12.0
T-904-01-265	7.1	3.6		7.1	12.0	0.0		12.0

Line	A13-1	A13-2	A13-3	A13 highest	A60-1	A60-2	A60-3	A60 highest
T-904-01-267	0.0	0.0	2.7	2.7	0.0	0.0	7.4	7.4
T-904-01-269	10.0	0.0		10.0	0.0	0.0	4.2	4.2
T-904-01-271	77.8	15.4		77.8	3.4	6.7		6.7
T-904-01-273	82.8	23.3		82.8	32.1	25.0		32.1
T-904-01-275	17.2	0.0		17.2	0.0	3.6	7.1	7.1
T-904-01-277	55.6	31.0		55.6	17.4	16.7		17.4
T-904-01-279	14.3	0.0		14.3	0.0	0.0	3.7	3.7
T-904-01-281	10.3	0.0		10.3	0.0	0.0	0.0	0.0
T-904-01-283	36.7	17.9		36.7	0.0	0.0	33.3	33.3
T-904-01-293	58.3	19.2		58.3	7.7	10.7		10.7
T-904-01-295	34.6	0.0		34.6	17.9	3.6		17.9
T-904-01-297	26.7	13.8		26.7	0.0	3.4	13.8	13.8
T-904-01-299	18.5	0.0		18.5	0.0	0.0	13.8	13.8
T-904-01-301	25.0	3.6		25.0	7.1	4.0		7.1
T-904-01-303	20.7	10.0		20.7	3.7	10.7		10.7
T-904-01-305	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T-904-01-307	44.0	0.0		44.0	0.0	0.0	4.0	4.0
T-904-01-309	78.6	20.0		78.6	11.1	3.8		11.1
T-904-01-311	42.9	12.5		42.9	10.3	7.1		10.3
T-904-01-313	29.6	26.7		29.6	11.5	3.8		11.5
T-904-01-315	53.6	30.0		53.6	7.4	10.0		10.0
T-904-01-317	18.5	0.0		18.5	0.0	0.0	0.0	0.0
T-904-01-319	4.2	3.6	0.0	4.2	0.0	0.0	0.0	0.0
T-904-01-321	7.7	0.0		7.7	0.0	0.0	7.1	7.1
T-904-01-323	34.5	17.9		34.5	11.1	6.9		11.1
T-904-01-325	20.0	0.0		20.0	0.0	0.0	3.8	3.8
T-904-01-327	23.3	13.8		23.3	0.0	0.0	3.8	3.8
T-904-01-329	8.7	0.0		8.7	0.0	7.7		7.7
T-904-01-331	18.2	20.7		20.7	7.4	16.7		16.7
T-904-01-333	3.7	0.0	13.6	13.6	0.0	0.0	0.0	0.0
T-904-01-335	77.8	24.1		77.8	14.3	14.3		14.3

Line	A13-1	A13-2	A13-3	A13 highest	A60-1	A60-2	A60-3	A60 highest
T-904-01-337	9.1	0.0		9.1	3.6	3.8	4.3	4.3
T-904-01-339	86.2	33.3		86.2	7.1	20.7		20.7
T-904-01-341	25.9	3.4		25.9	0.0	0.0	3.7	3.7
T-904-01-343	40.0	0.0		40.0	4.0	6.7		6.7
T-904-01-345	17.2	0.0		17.2	0.0	3.6	4.3	4.3
T-904-01-347	0.0	0.0		0.0	0.0	0.0	0.0	0.0
T-904-01-349	29.6	0.0		29.6	3.8	3.4	0.0	3.8
T-904-01-351	10.7	12.5		12.5	0.0	3.6	20.8	20.8
T-904-01-353	16.7	3.4		16.7	0.0	0.0	3.6	3.6
T-904-01-355	46.7	57.1		57.1	7.7	9.5		9.5
CDC Dancer	3.7	0.0	0.0	3.7		0.0	0.0	0.0
AC Morgan	75.0	27.6	4.3	75.0		18.5	28.6	28.6
Hazel	64.3	42.3	0.0	64.3		0.0	0.0	0.0
Belle	52.6	3.6	5.7	52.6		6.3	3.8	6.3
Ogle	66.7	33.3	8.8	66.7		0.0	0.0	0.0
Starter	4.0	0.0	21.1	21.1		3.6	0.0	3.6
PY11108	92.0	38.5	0.0	92.0		0.0	4.5	4.5

Line	A617-1	A617-2	A617-3	A617 highest
T-904-01-005	0.0	0.0	3.6	3.6
T-904-01-007	16.0	7.7		16.0
T-904-01-009	0.0	0.0	3.4	3.4
T-904-01-011	0.0	20.8		20.8
T-904-01-013	20.0	25.0		25.0
T-904-01-015	10.3	27.6		27.6
T-904-01-017	17.9	7.7		17.9
T-904-01-019	0.0	0.0	3.6	3.6
T-904-01-021	28.6	14.8		28.6
T-904-01-023	35.7	16.0		35.7
T-904-01-025	0.0	3.8	12.0	12.0
T-904-01-027	3.8	0.0	0.0	3.8
T-904-01-029	3.4	0.0	0.0	3.4
T-904-01-031	0.0	23.5		23.5
T-904-01-033	38.9	0.0		38.9
T-904-01-035	4.0	8.0		8.0
T-904-01-037	0.0	0.0	3.7	3.7
T-904-01-039	0.0	10.7		10.7
T-904-01-041	16.0	16.7		16.7
T-904-01-043	22.2	40.0		40.0
T-904-01-045	0.0	0.0	0.0	0.0
T-904-01-047	0.0	0.0	3.4	3.4
T-904-01-049	0.0	0.0	21.4	21.4
T-904-01-051	66.7	59.1		66.7
T-904-01-053	40.7	50.0		50.0
T-904-01-055	10.0	14.3		14.3
T-904-01-057	20.7	6.3		20.7
T-904-01-059	3.8	54.5		54.5
T-904-01-061	69.0	11.5		69.0
T-904-01-063	17.9	16.7		17.9
T-904-01-065	55.2	0.0		55.2

Line	A617-1	A617-2	A617-3	A617 highest
T-904-01-067	34.5	20.8		34.5
T-904-01-069	35.7			35.7
T-904-01-071	0.0	0.0	16.7	16.7
T-904-01-073	0.0	0.0	3.4	3.4
T-904-01-075	0.0	3.6	3.6	3.6
T-904-01-077	0.0	7.4		7.4
T-904-01-079	24.1	6.7		24.1
T-904-01-081	44.8	46.2		46.2
T-904-01-083	53.3	62.1		62.1
T-904-01-085	16.0	9.5		16.0
T-904-01-087	51.9	6.9		51.9
T-904-01-089	33.3	39.3		39.3
T-904-01-091	22.2	6.7		22.2
T-904-01-101	0.0	0.0		0.0
T-904-01-103	0.0	0.0	14.3	14.3
T-904-01-105	11.5	14.8	3.4	14.8
T-904-01-107	36.0	23.8		36.0
T-904-01-109	3.6	0.0	14.3	14.3
T-904-01-111	13.0	3.8		13.0
T-904-01-113	23.1	30.4		30.4
T-904-01-115	26.1	11.1		26.1
T-904-01-117	22.2	48.3		48.3
T-904-01-119	6.9	8.7		8.7
T-904-01-121	0.0	0.0	0.0	0.0
T-904-01-123	0.0	0.0	0.0	0.0
T-904-01-125	70.0	45.8		70.0
T-904-01-129	48.1	22.2		48.1
T-904-01-131	14.3	0.0		14.3
T-904-01-133	4.0	15.4		15.4
T-904-01-135	17.2	12.0		17.2
T-904-01-137	0.0	0.0	0.0	0.0

Line	A617-1	A617-2	A617-3	A617 highest
T-904-01-139	0.0	0.0	0.0	0.0
T-904-01-141	0.0	7.4		7.4
T-904-01-143	34.5	10.3		34.5
T-904-01-145	48.0	61.9		61.9
T-904-01-147	0.0	4.2	13.0	13.0
T-904-01-149	0.0	4.0	0.0	4.0
T-904-01-151	3.6	5.3		5.3
T-904-01-153	51.7	38.5		51.7
T-904-01-155	35.7	19.2		35.7
T-904-01-157	4.2	3.7	20.7	20.7
T-904-01-159	0.0	0.0	3.8	3.8
T-904-01-161	0.0	0.0	0.0	0.0
T-904-01-163	68.0	36.0		68.0
T-904-01-165	30.4	20.0		30.4
T-904-01-167	0.0	4.3	7.1	7.1
T-904-01-169	18.5	23.1		23.1
T-904-01-171	0.0	0.0	3.4	3.4
T-904-01-175	4.0	23.5		23.5
T-904-01-177	17.2	7.4		17.2
T-904-01-179	0.0	0.0	0.0	0.0
T-904-01-181	0.0	0.0	0.0	0.0
T-904-01-183	60.0	59.3		60.0
T-904-01-185	0.0	0.0	7.7	7.7
T-904-01-187	0.0	0.0	0.0	0.0
T-904-01-197	0.0	0.0	7.1	7.1
T-904-01-199	3.4	0.0	6.9	6.9
T-904-01-201	7.4	0.0		7.4
T-904-01-203	0.0	0.0	0.0	0.0
T-904-01-205	0.0	0.0		0.0
T-904-01-207	15.4	0.0	13.3	15.4
T-904-01-211	3.6	#VALUE!	4.0	4.0

Line	A617-1	A617-2	A617-3	A617 highest
T-904-01-213	15.4	10.0		15.4
T-904-01-215	0.0	0.0	0.0	0.0
T-904-01-217	4.5	4.3	19.0	19.0
T-904-01-219	8.7	4.5		8.7
T-904-01-221	0.0	4.3	4.5	4.5
T-904-01-223	12.0	18.2		18.2
T-904-01-225	0.0	0.0	0.0	0.0
T-904-01-227	4.0	0.0	0.0	4.0
T-904-01-229	15.4	39.3		39.3
T-904-01-231	8.0	24.0		24.0
T-904-01-233	0.0	3.7	4.5	4.5
T-904-01-235	0.0	4.8	0.0	4.8
T-904-01-237	0.0	4.3	6.9	6.9
T-904-01-239	0.0	0.0	0.0	0.0
T-904-01-241	10.7	16.7		16.7
T-904-01-243	48.3	7.4		48.3
T-904-01-245	3.6	24.0		24.0
T-904-01-247	24.1	0.0		24.1
T-904-01-251	48.0	17.4		48.0
T-904-01-253	7.4	9.1		9.1
T-904-01-255	11.1	37.5		37.5
T-904-01-257	0.0	0.0	8.0	8.0
T-904-01-259	3.8	10.3		10.3
T-904-01-261	0.0	4.3	4.3	4.3
T-904-01-263	11.1	31.8		31.8
T-904-01-265	10.3	4.0		10.3
T-904-01-267	0.0	0.0	14.3	14.3
T-904-01-269	0.0	4.0	0.0	4.0
T-904-01-271	23.1	21.4		23.1
T-904-01-273	51.7	72.7		51.7
T-904-01-275	0.0	9.5		9.5

Line	A617-1	A617-2	A617-3	A617 highest
T-904-01-277	22.7	11.8		22.7
T-904-01-279	0.0	0.0	4.3	4.3
T-904-01-281	0.0	6.7		6.7
T-904-01-283	21.4	0.0		21.4
T-904-01-293	39.3	7.1		39.3
T-904-01-295	34.8	30.0		34.8
T-904-01-297	3.4	17.4		17.4
T-904-01-299	0.0	4.8	7.7	7.7
T-904-01-301	13.3	0.0		13.3
T-904-01-303	3.8	20.7		20.7
T-904-01-305	0.0	0.0	0.0	0.0
T-904-01-307	0.0	0.0	21.4	21.4
T-904-01-309	29.2	8.0		29.2
T-904-01-311	12.0	25.0		25.0
T-904-01-313	40.0	16.7		40.0
T-904-01-315	4.5	41.4		41.4
T-904-01-317	0.0	0.0	4.8	4.8
T-904-01-319	0.0	0.0	0.0	0.0
T-904-01-321	0.0	8.3		8.3
T-904-01-323	3.7	0.0	25.0	25.0
T-904-01-325	0.0	0.0	0.0	0.0
T-904-01-327	3.4	0.0	14.8	14.8
T-904-01-329	0.0	0.0	3.4	3.4
T-904-01-331	7.4	29.6		29.6
T-904-01-333	3.6	0.0	0.0	3.6
T-904-01-335	26.9	25.0		26.9
T-904-01-337	0.0	0.0	0.0	0.0
T-904-01-339	70.4	51.9		70.4
T-904-01-341	0.0	11.1		11.1
T-904-01-343	7.4	17.4		17.4
T-904-01-345	0.0	3.6	6.9	6.9

Line	A617-1	A617-2	A617-3	A617 highest
T-904-01-347	0.0	4.8	4.5	4.8
T-904-01-349	0.0	0.0	0.0	0.0
T-904-01-351	8.7	4.8		8.7
T-904-01-353	0.0	4.2	0.0	4.2
T-904-01-355	50.0	20.0		50.0
CDC Dancer	0.0	0.0	0.0	0.0
AC Morgan	17.9	44.0	50.0	50.0
Hazel	25.0	46.4	57.1	57.1
Belle	6.3	37.5	12.5	37.5
Ogle	63.3	50.0	51.9	63.3
Starter	0.0	0.0	0.0	0.0
PY11108	22.7	13.3	18.2	22.7

Line	MN '13	MN '14	MN '15	MN highest	SKTN '15	SKTN highest
T-904-01-005	1	0	0	5		10
	0	0	5		10	
T-904-01-007	40	1	15	40		30
	15	5	20		30	
T-904-01-009	1	0	0	1	2	3
	1		1		3	
T-904-01-011	40	10	30	40	70	70
	40		40		40	
T-904-01-013	5	5	5	10	30	30
	10	0	10		15	
T-904-01-015	20	5	25	25	40	70
	20	10	25		70	
T-904-01-017	30	10	20	40	60	60
	40	10	40		50	
T-904-01-019	5	0	5	15	0	1
	1	0	15		1	
T-904-01-021	50	10	30	50	40	50
	40	10	30		50	
T-904-01-023	40	5	20	50	50	50
	50	5	25		40	
T-904-01-025	5	1	5	20	20	20
	5	0	20			
T-904-01-027	1	0	1	1		.
	1	0	1			
T-904-01-029	5	0	0	5	2	2
	1	0	5		2	
T-904-01-031	5	5	10	15		
	5	0	15		15	
T-904-01-033	20	0	25	40	40	40
	20	5	40		20	
T-904-01-035	15	0	15	20	10	10
	10	1	20			

Line	MN '13	MN '14	MN '15	MN highest	SKTN '15	SKTN highest
T-904-01-037	15	0		20	10	10
	0		20			
T-904-01-039	5	0	5	10	4	10
	1	0	10		10	
T-904-01-041	10	0	15	20	15	25
	5	0	20		25	
T-904-01-043	40	5	25	40	30	30
	15	5	40			
T-904-01-045	0	0	1	1	0	0
	1	0	1			
T-904-01-047	1	0	10	15	10	10
	5	1	15			
T-904-01-049	10	1	15	20	30	30
	10	5	20			
T-904-01-051	40	15	60	70		
	50	10	70		80	
T-904-01-053	40	10	40	50	70	70
	30	10	50		30	
T-904-01-055	40	5	30	40	70	70
	20	15	30		30	
T-904-01-057	50	5	30	50	40	40
	15	5	30			
T-904-01-059	15	5	15	20	25	25
	20	0	20		25	
T-904-01-061	50	25	60	70	70	70
	60	20	70		70	
T-904-01-063	10	1	10	20	15	20
	10	0	20		20	
T-904-01-065	70	20	50	70	80	80
	70	15	50			
T-904-01-067	30	15	20	40		30
	40	0	30		30	
T-904-01-069	30	10	20	40	50	50

Line	MN '13	MN '14	MN '15	MN highest	SKTN '15	SKTN highest
T-904-01-069	15	5	40		50	
T-904-01-071	5	1	15	15	3	10
	5	0	15		10	
T-904-01-073	1	0	0	10	2	3
	1	0	10		3	
T-904-01-075	5	1		15	10	15
	5	0	15		15	
T-904-01-077	5	5	10	10	4	10
	5	0			10	
T-904-01-079	10	10	15	15	50	50
	15	5			40	
T-904-01-081	50	25	40	60	50	50
	60				30	
T-904-01-083	30	10	40	40	50	50
	15	0				
T-904-01-085	10	0	15	20	10	15
	15	5	20		15	
T-904-01-087	40	1	40	50	80	80
	30	10	50			
T-904-01-089	40	10	25	40	60	60
	40	15	30			
T-904-01-091	30	5	40	50	50	50
	50	10	40		20	
T-904-01-101	15	5	20	30	10	15
	5	0	30		15	
T-904-01-103	5	1	5	5	3	4
	1	0	5		4	
T-904-01-105	15	10	25	40	50	70
	40	20	30		70	
T-904-01-107	40	15	20	50	50	50
	50	10	30		25	
T-904-01-109	10	0	10	25	10	15
	10	5	25		15	

Line	MN '13	MN '14	MN '15	MN highest	SKTN '15	SKTN highest
T-904-01-111	10	1	10	15	10	10
	10	1	15		10	
T-904-01-113	15	10	15	30	40	40
	30	5	20			
T-904-01-115	15	5	15	40	10	10
	40	5	25			
T-904-01-117	50	15	30	50	70	70
	50	15	40		40	
T-904-01-119	5	1	10	10	4	4
	5	0	10			
T-904-01-121	5	0	5	15	0	5
	5	0	15		5	
T-904-01-123	1	0	5	5	2	2
	1	0	5		1	
T-904-01-125	60	10	60	70	70	80
	70	20	70		80	
T-904-01-129					50	50
					50	
T-904-01-131	5	0	15	15	10	25
	10		15		25	
T-904-01-133	10	5	15	20	15	15
	5	5	20		15	
T-904-01-135	10	5	15	30	25	30
	10	10	30		30	
T-904-01-137	5	0	0	5		2
	1	10	1		2	
T-904-01-139	1	0	0	1	1	1
	1	0	0		0	
T-904-01-141	5	1	5	5	20	20
	1		5			
T-904-01-143	30	10	20	40	40	60
	20	1	40		60	
T-904-01-145	50	20	30	50	70	70

Line	MN '13	MN '14	MN '15	MN highest	SKTN '15	SKTN highest
T-904-01-145	50	5	40			
T-904-01-147	5	1	15	15	0	3
	1	0	15		3	
T-904-01-149	5	0	5	10	10	10
	1	0	10			
T-904-01-151	1	0	5	5	10	10
	1		5			
T-904-01-153	40	10	15	40	40	40
	40	10	30			
T-904-01-155	15	1	30	30	20	40
	20	5	30		40	
T-904-01-157	15	5	10	20	10	10
	15	0	20		10	
T-904-01-159	1	0	5	5	2	10
	1	0	5		10	
T-904-01-161	10	0	5	15		10
	5	5	15		10	
T-904-01-163	50	10	30	60	50	50
	30		60		40	
T-904-01-165	50	10	25	50	40	50
	30	10			50	
T-904-01-167	10	0	10	10	15	15
	5	5				
T-904-01-169	20	5	25	25	30	30
	10	5				
T-904-01-171	5	0	5	5	10	10
	1	0			3	
T-904-01-175	5	0	15	15	30	30
	0	1			15	
T-904-01-177	10	0	10	25	20	40
	5	0	25		40	
T-904-01-179	1	0	5	15	3	4
	0	0	15		4	

Line	MN '13	MN '14	MN '15	MN highest	SKTN '15	SKTN highest
T-904-01-181	5	0	5	15	10	10
	1	0	15			
T-904-01-183	60	10	30	60	80	80
	50	5	50		25	
T-904-01-185	5	1	5	15	7	7
	1	0	15		1	
T-904-01-187	1	0	1	5	1	1
	1	0	5		0	
T-904-01-197	1	0	10	15	15	15
	1	0	15		10	
T-904-01-199	0	0	5	15	5	10
	0	0	15		10	
T-904-01-201	5	0	1	10		3
	0	0	10		3	
T-904-01-203	1	0	0	1	0	1
	1	0	0		1	
T-904-01-205	1	0	0	1	2	3
	0	0	0		3	
T-904-01-207	15	0	15	15	30	30
	10	5	15		15	
T-904-01-211	5	0	5	10	10	10
	1	0	10		10	
T-904-01-213	15	0	15	15	20	20
	10	5	15		20	
T-904-01-215	15	0	5	15	5	5
	1	0	5		3	
T-904-01-217	10	5	15	15		15
	1	5	15		15	
T-904-01-219		0	15	20	40	40
	15	5	20		30	
T-904-01-221		0	20	20	30	40
	10	1			40	
T-904-01-223	30	5	20	30	40	50

Line	MN '13	MN '14	MN '15	MN highest	SKTN '15	SKTN highest
T-904-01-223	10	1	30		50	
T-904-01-225	1	0	1	5	10	10
	0		5		3	
T-904-01-227	0	0	0	5	5	5
	0	0	5		2	
T-904-01-229	15	0	20	40	15	15
	1	5	40		15	
T-904-01-231	10	5	10	20	15	15
	10	5	20		15	
T-904-01-233	1	0	1	10	2	3
	1	0	10		3	
T-904-01-235	5	0	1	10	0	0
	1	0	10			
T-904-01-237	5	5	15	15	10	10
	5	0	15		10	
T-904-01-239	1	0	0	1	3	10
	0	0	0		10	
T-904-01-241	15	5	15	15		40
	10	5	15		40	
T-904-01-243	30	1	30	40	50	50
	40	5	40		40	
T-904-01-245	10	1	20	20		25
	1	5	20		25	
T-904-01-247	5	5	15	15	10	10
	5	0	15			
T-904-01-251	30	10	20	30	30	40
	30	10	20		40	
T-904-01-253	5	1	5	10	1	10
	1	5	10		10	
T-904-01-255	10	1		15	20	30
	10	5	15		30	
T-904-01-257	0	0	1	5	3	3
	0	1	5			

Line	MN '13	MN '14	MN '15	MN highest	SKTN '15	SKTN highest
T-904-01-259	15	0	10	40	25	25
	15	1	40		25	
T-904-01-261	10	1	1	10	0	0
	1	0	10			
T-904-01-263	30	5	25	40	50	50
	40	5			40	
T-904-01-265	1	5	10	15		15
	1	0	15		15	
T-904-01-267	0	0	1	15	0	4
	0	0	15		4	
T-904-01-269	1	0	0	5	3	15
	0	0	5		15	
T-904-01-271	20	15	20	40	50	50
	20	1	40		25	
T-904-01-273	60	10	50	70	70	70
	70	15	70			
T-904-01-275	1	0	1	10	5	10
	1	1	10		10	
T-904-01-277	40	10	30	60		25
	30	10	60		25	
T-904-01-279	0	0	0	1	3	3
	0	1	1			
T-904-01-281	1	0	5	10	0	0
	1	0	10			
T-904-01-283	5	0	20	25	40	40
	10		25		20	
T-904-01-293	30	5	30	50	50	50
	20	1	50			
T-904-01-295	50	15	40	50	60	60
	15	10			50	
T-904-01-297	10	10	20	20	60	60
	15	5	20		15	
T-904-01-299	5	1	1	5	10	10

Line	MN '13	MN '14	MN '15	MN highest	SKTN '15	SKTN highest
T-904-01-299	1	0	5			
T-904-01-301	0	1	0	5	5	5
	1	1	5		5	
T-904-01-303	1	10	5	10	15	15
	5	5	10		15	
T-904-01-305	1	0	0	1	0	0
	0	0	1			
T-904-01-307	5	0	5	20	20	25
	0	0	20		25	
T-904-01-309	20	5	25	40		50
	10	5	40		50	
T-904-01-311	20	0	15	20		60
	20	1	20		60	
T-904-01-313	15	0	25	40	30	30
	30	5	40		30	
T-904-01-315	20	5	30	40	40	50
	20	10	40		50	
T-904-01-317	5	0	0	5	2	6
	1	0	0		6	
T-904-01-319	1	0	1	5		1
	5	0	1		1	
T-904-01-321	1	0	0	1	3	3
	1	0	1			
T-904-01-323	15	5	15	15	30	30
	15	5	15		20	
T-904-01-325	0	0	5	10	4	4
	0	0	10		1	
T-904-01-327	10	0	5	10	15	15
	0	0	10		10	
T-904-01-329	10	0	0	10	0	1
	1	0	0		1	
T-904-01-331	10	5	20	25	50	50
	20	5	25		50	

Line	MN '13	MN '14	MN '15	MN highest	SKTN '15	SKTN highest
T-904-01-333	5	0	5	5	3	3
	1	0	5		2	
T-904-01-335	40	15	30	40	50	60
	40		50		60	
T-904-01-337	5	0	0	5	5	5
	5	0	1			
T-904-01-339	50	15	30	60	60	60
	60	30	40			
T-904-01-341	5	5		10		5
	5	5	10		5	
T-904-01-343	10	5		15	10	10
	5	5	15		10	
T-904-01-345	10	5		15	1	1
	5	5	15			
T-904-01-347	5	0		5	3	3
	5	0	0			
T-904-01-349	1	0	1	15	7	7
	0	1	15		1	
T-904-01-351	10	1	15	15	15	15
	5	5	15		10	
T-904-01-353	5	0	10	10	3	3
	5	1	10			
T-904-01-355	70	5	40	70	80	80
	70	1	60		80	
CDC Dancer	5	0	0	5	2	10
	1	0	0		10	
	1		0			
	1		0			
	1		0			
	1		1			
	1		5			
	1					
	1					

Line	MN '13	MN '14	MN '15	MN highest	SKTN '15	SKTN highest
CDC Dancer	1					
	5					
	5					
AC Morgan	20	5	15	40	20	20
	40	5	20			
	30		20			
	15		20			
	20		25			
	15		25			
	15		30			
	20					
	20					
	15					
	30					
	10					
Hazel	40	50	40	70	80	80
	50	50	40		80	
	40	50	40			
	50	50	50			
		50	50			
		50	60			
		70	60			
		60				
Belle	10	5	0	15	30	30
	5	5	0		10	
	10	15	5			
	5	5	10			
		10	10			
		0	10			
		10	10			
		15				
Ogle	50	60	60	90	30	30
	60	80	60			

Line	MN '13	MN '14	MN '15	MN highest	SKTN '15	SKTN highest
Ogle	60	80	60			
	70	70	70			
		70	70			
		80	70			
		90	80			
		90	80			
Starter	1	0	0	10	5	5
	0	0	1		0	
	0	1	1			
	5	0	5			
		1	5			
		5	10			
		10	10			
		5	10			
PY11108	90	90	60	90	70	70
	90	90	60			
	90	80	80			
	90	80	80			
		90	90			
		90	90			
		90	90			
		90				

Appendix C
Linkage Map of the CDC Dancer x AC Morgan Population

Table C1. Genetic map positions for the 737 SNP markers mapped to 34 linkage groups in the CDC Dancer x AC Morgan population.

Linkage Group 1		Linkage Group 2		Linkage Group 3		Linkage Group 4	
Marker Name	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**
Gmi_ds_a3_319_180	0	Gmi_gbs_24062	0	Gmi_ds_lb_10188	0	Gmi_es_lb_8077	0
Gmi_ds_lb_10176	1.08	Gmi_es15_c497_486	1.89	Gmi_ds_lb_10187	0	Gmi_es02_c3016_666	6.38
Gmi_ds_lb_6584	10.95	Gmi_es03_c1628_303	8.97	Gmi_gbs_116364	0	Gmi_gbs_81931	8.56
Gmi_es02_c933_584	12.79	Gmi_es14_c1496_463	8.97	Gmi_gbs_43789	0	Gmi_es02_c10035_217	36.02
Gmi_es05_c5751_396	18.01	Gmi_es14_c2632_611	8.97	Gmi_es05_c9987_517	2.8	Gmi_es15_c5060_161	36.74
Gmi_es05_lrc23969_245	19.4	Gmi_es05_c13035_138	10.1	Gmi_gbs_112216	6.98	Gmi_es02_c4865_190	36.74
Gmi_es05_c15094_281	19.74	Gmi_es_lb_3620	10.1	Gmi_gbs_8948	8.13	Gmi_es01_c8255_502	37.08
Gmi_es01_c515_543	20.07	Gmi_es17_c1288_844	10.1	Gmi_es01_c20473_219	8.48	Gmi_gbs_105875	43.85
Gmi_es05_lrc9304_238	21.47	Gmi_es03_c1554_544	10.1	Gmi_es_lb_11672	8.87	Gmi_es01_c9955_227	44.21
Gmi_ds_cc7543_80	40.62	Gmi_es17_c3218_276	10.1			Gmi_es01_c20367_331	44.21
Gmi_ds_cc7482_102	40.62	Gmi_es14_c6336_208	10.1			Gmi_ds_lb_10786	44.56
Gmi_ds_lb_10262	40.62	Gmi_es02_c31310_407	10.1			Gmi_gbs_101811	44.56
Gmi_es03_c1515_642	42.05	Gmi_es22_c8027_128	10.1			Gmi_es14_c18975_277	44.56
Gmi_es15_c6974_369	42.05	Gmi_es_cc15368_155	10.1			Gmi_es17_c16539_472	44.56
Gmi_es02_lrc23397_543	62.57	Gmi_es02_c3020_292	10.1			Gmi_gbs_6872	44.89
Gmi_ds_lb_6442	62.57	Gmi_es22_c15684_157	10.1			Gmi_es02_lrc27323_95	44.89
Gmi_es15_c6161_133	62.57	Gmi_es01_c1475_450	10.1			Gmi_gbs_70578	45.23
		Gmi_es_lb_9892	10.1			Gmi_es_lb_11602	45.23
		Gmi_es_lb_9187	10.1			Gmi_es03_c18720_462	45.23
		Gmi_ds_lb_3438	10.1			Gmi_gbs_58282	45.23
		Gmi_es03_c6926_224	10.1			Gmi_es05_c26171_192	45.23
		Gmi_es01_lrc21199_411	10.1			Gmi_es22_c20350_257	45.23
		Gmi_es17_c2162_547	10.1			Gmi_ds_lb_10400	45.58
		Gmi_es01_c17597_189	10.1			Gmi_es22_c20081_313	45.58
		Gmi_es02_c3764_274	10.1			Gmi_ds_lb_4609	45.58
		Gmi_ds_cc8829_137	10.1			Gmi_es17_lrc19617_111	45.58

Linkage Group 1		Linkage Group 2		Linkage Group 3		Linkage Group 4	
Marker Name	Pos	Marker Name	Pos	Marker Name	Pos	Marker Name	Pos
		Gmi_es05_c2330_370	10.1			Gmi_gbs_109589	45.58
		Gmi_es17_c2137_336	10.1			Gmi_es17_c4148_611	45.58
		Gmi_ds_lb_5489	10.1			Gmi_gbs_71132	45.58
		Gmi_gbs_8790	10.1			Gmi_gbs_79375	46.26
		Gmi_es01_c25788_216	10.1			Gmi_es03_c5837_61	46.26
		Gmi_ds_lb_5621	10.1			Gmi_es02_c19514_881	46.61
		Gmi_es17_c2744_673	10.1			Gmi_es01_c9377_521	46.61
		Gmi_es17_c17781_268	10.1			Gmi_es12_c8736_490	46.61
		Gmi_es15_c6587_292	10.1			Gmi_ds_lb_10721	46.61
		Gmi_es17_c4223_141	10.1			Gmi_ds_lb_8677	46.61
		Gmi_ds_cc5188_127	10.1			Gmi_es22_c9270_194	46.61
		Gmi_ds_lb_1721	10.1			Gmi_es01_c1820_583	46.61
		Gmi_es03_c5236_182	10.1			Gmi_ds_cc8539_101	46.61
		Gmi_es17_c1732_618	10.1			Gmi_es02_c17906_415	47.73
		Gmi_gbs_88885	10.1			Gmi_ds_lb_3488	49.62
		Gmi_es17_c2669_776	10.1			Gmi_es15_c32_761	49.98
		Gmi_es22_c18772_417	10.1			Gmi_es02_c14080_371	49.98
		Gmi_es07_c15735_587	10.1			Gmi_es17_c9023_409	49.98
		Gmi_es17_c10413_657	10.1			Gmi_es05_lrc18884_211	50.34
		Gmi_es01_c11420_274	10.1			Gmi_es_cc7714_103	50.34
		Gmi_es01_c15859_246	10.1			Gmi_es17_c13962_600	50.72
		Gmi_es03_c9527_76	10.1			Gmi_es22_c2904_356	51.09
		Gmi_gbs_61514	10.79			Gmi_es05_c3072_662	51.09
		Gmi_es02_c9694_350	10.79			Gmi_es03_c19505_223	51.09
		Gmi_es17_c962_684	10.79			Gmi_es05_c21329_243	52.52
		Gmi_es17_c7387_367	10.79			Gmi_es02_c23166_443	52.87
		Gmi_es14_c1182_796	15.09			Gmi_ds_lb_6840	55.81
		Gmi_es15_c7819_478	24.2			Gmi_ds_lb_6609	55.81
		Gmi_ds_opt_14877_117	24.56			Gmi_es01_c16727_290	56.5
		Gmi_es_lb_9789	26.49			Gmi_es01_c22540_100	56.5

Linkage Group 1		Linkage Group 2		Linkage Group 3		Linkage Group 4	
Marker Name	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**
		Gmi_es02_c12942_675	26.49			Gmi_gbs_86839	56.85
		Gmi_es17_c3660_457	29.03			Gmi_es15_c14533_341	58.28
		Gmi_es17_c3660_713	29.03			Gmi_ds_lb_6383	60.04
		Gmi_es02_c16953_600	30.11			Gmi_es17_c3583_265	61.87
		Gmi_gbs_70344	43.61			Gmi_ds_lb_6613	61.87
						Gmi_ds_lb_6613	61.87
						Ba_grs_c10318_236	64.21
						Gmi_es01_c8788_182	64.57

Linkage Group 5		Linkage Group 6		Linkage Group 7		Linkage Group 8	
Marker Name	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**
Gmi_es02_c11450_462	0	Gmi_ds_lb_3563	0	Gmi_es03_c17575_451	0	Gmi_es02_lrc28702_171	0
Gmi_ds_lb_1995	0.34	Gmi_es15_c8662_158	0	Gmi_es17_c793_567	1.87	Gmi_es01_c11741_182	0
Gmi_gbs_46202	0.34	Gmi_ds_cc8262_71	12.65	Gmi_ds_lb_321	1.87	Gmi_es02_c25482_475	0
Gmi_es_lb_11741	1.03	Gmi_es03_c695_117	13.32	Gmi_es15_c10103_419	1.87	Gmi_gbs_78834	0
Gmi_gbs_83715	1.03	Gmi_ds_lb_5450	13.99	Gmi_es05_c8058_436	2.94	Gmi_es01_c23001_372	0
Gmi_ds_a3_340_378	2.7	Gmi_es01_c13259_737	13.99	Gmi_es01_c2742_497	3.31	Gmi_es17_c6637_470	1.1
Gmi_ds_lb_4867	2.7	Gmi_es11_c2305_1071	13.99	Gmi_ds_cc6822_86	4.84	Gmi_ds_cc5751_111	1.46
Gmi_gbs_112167	4.05	Gmi_es02_c5306_435	13.99	Gmi_es17_c3006_879	4.84	Gmi_gbs_113531	1.46
Gmi_gbs_81501	5.07	Gmi_es22_c4593_1054	32.94	Gmi_es22_lrc16709_212	5.93	Gmi_es_cc12905_159	1.46
Gmi_es22_c9147_376	5.41	Gmi_ds_lb_1611	32.94	Gmi_es22_c11452_253	7.02	Gmi_es02_c13608_538	1.82
Gmi_gbs_35330	5.41	Gmi_ds_lb_2087	35.8	Gmi_es15_c3296_521	7.02	Gmi_es02_c2276_311	1.82
Gmi_es05_c22725_367	7.75	Gmi_es_cc15237_134	35.8	Gmi_es15_c3003_520	7.38	Gmi_ds_cc5134_229	2.53
Gmi_es03_c9483_351	7.75	Gmi_ds_lb_7112	35.8	Gmi_es17_c13210_256	7.38	Gmi_es05_c20438_78	2.53
Gmi_es02_c14249_794	8.42	Gmi_es14_c12998_449	36.87	Gmi_es22_c20794_312	8.47	Gmi_es01_c461_1288	2.53
				Gmi_es02_c3374_73	8.47	Gmi_es17_c2398_610	2.53
				Gmi_es_lb_8042	8.47	Gmi_es17_c5784_752	2.53
				Gmi_es02_c7654_733	8.47	Gmi_es_cc15057_51	2.53
				Gmi_es03_c1325_823	8.47	Gmi_es14_c9543_464	3.97
				Gmi_es14_c6848_443	8.47	Gmi_ds_cc1149_344	4.68
				Gmi_ds_lb_95	8.47	Gmi_es05_c1774_174	8.72
				Gmi_gbs_89190	8.47	Gmi_gbs_69683	10.24
				Gmi_es05_c1910_886	8.47	Gmi_es03_c6194_428	10.6
				Gmi_gbs_37983	8.47	Gmi_es15_c1433_87	14.75
				Gmi_es15_c1917_488	8.47	Gmi_ds_cc4376_194	16.92
				Gmi_es17_c9476_330	8.47	Gmi_es02_c4520_381	21.66
				Gmi_es02_c13236_178	8.47	Gmi_gbs_27675	25.24
				Gmi_es01_c16767_69	8.47	Gmi_es17_c20203_300	25.62
				Gmi_es22_c1562_103	8.47		
				Gmi_es17_c6653_226	8.47		

Linkage Group 5		Linkage Group 6		Linkage Group 7		Linkage Group 8	
Marker Name	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**
				Gmi_ds_lb_7139	8.47		
				Gmi_gbs_9614	8.83		
				Gmi_es22_c8005_394	9.19		
				Gmi_es17_c11616_253	9.19		
				Gmi_es05_c26190_676	9.19		
				Gmi_es03_lrc10769_351	9.19		
				Gmi_es03_c13946_240	9.19		
				Gmi_es02_c2221_329	9.19		
				Gmi_es15_c6458_250	9.88		
				Gmi_es05_c11331_441	9.88		
				Gmi_es15_c1604_447	10.63		
				Gmi_es11_c21345_385	10.63		
				Gmi_ds_lb_5673	11.75		
				Gmi_gbs_53244	11.75		
				Gmi_ds_lb_10925	12.13		
				Gmi_es15_c19863_323	12.5		
				Gmi_es02_c2752_372	12.5		
				Gmi_es05_c18726_496	12.88		
				Gmi_es15_c1324_755	35.61		
				Gmi_es03_c21572_224	45.48		
				Gmi_es22_c21243_266	45.84		
				Gmi_gbs_17297	48.06		
				Gmi_es17_c467_205	48.06		
				Gmi_ds_lb_1757	48.06		
				Gmi_es17_c5049_177	49.78		
				Gmi_es17_c12954_551	49.78		
				Gmi_es17_c12954_551	49.78		
				Gmi_es17_c12954_551	49.78		
				Gmi_es15_c349_532	54.56		
				Gmi_gbs_65960	56.36		

Linkage Group 5		Linkage Group 6		Linkage Group 7		Linkage Group 8	
Marker Name	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**
				Gmi_es03_c15096_232	56.36		
				Gmi_es15_c568_654	56.36		
				Gmi_es01_c8882_176	57.03		
				Gmi_es_cc7307_489	70.98		
				Gmi_es03_c19054_48	70.98		
				Gmi_es02_c12013_349	72.19		
				Gmi_es14_c13157_417	73.83		
				Gmi_gbs_114344	76.91		
				Gmi_es17_c2750_1001	82.01		

Linkage Group 9		Linkage Group 10		Linkage Group 11		Linkage Group 12	
Marker Name	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**
Gmi_es05_c5211_395	0	Gmi_es_lb_9918	0	Gmi_es17_c8444_506	0	Gmi_es_cc3011_421	0
Gmi_gbs_25112	0	Gmi_ds_lb_4781	0	Gmi_es15_c3362_506	0.79	Gmi_es15_c7755_266	6.9
Gmi_es02_c6122_167	8.77	Gmi_gbs_76988	0	Gmi_ds_lb_2041	13.18	Gmi_es17_c2276_224	6.9
Gmi_es17_c2107_189	12.08	Gmi_es22_c2410_401	0.35	Gmi_es01_c9910_138	13.18	Gmi_es_lb_7322	7.95
Gmi_gbs_90477	13.14	Gmi_es_cc7312_286	0.35	Gmi_es03_c2880_225	13.18	Gmi_es15_c17448_349	7.95
Gmi_ds_cc6354_109	15.81	Gmi_es01_c14606_61	0.35	Gmi_es03_c2880_225	13.18	Gmi_es17_c19933_225	7.95
Gmi_es15_c794_171	15.81	Gmi_es05_c9758_147	0.35	Gmi_es01_c16437_198	15.46	Gmi_gbs_88528	7.95
Gmi_es_lb_11401	15.81	Gmi_es17_c1173_635	0.35	Gmi_es14_c3581_722	24.22	Gmi_es02_c28150_198	7.95
Gmi_es02_c17553_371	15.81	Gmi_es02_c310_458	0.35	Gmi_es22_c9230_196	25.33	Gmi_es01_c6200_493	7.95
Gmi_gbs_28206	15.81	Gmi_es05_c22174_562	0.35	Gmi_es22_c5925_216	26.45	Gmi_gbs_9578	7.95
Gmi_gbs_1994	16.16	Gmi_es22_c5422_320	4.08	Gmi_es_cc6557_563	26.45	Gmi_es15_c8008_218	7.95
Gmi_es15_c2046_330	16.16	Gmi_es05_c2253_434	4.43	Gmi_es02_c17403_691	27.66	Gmi_es17_c1242_703	7.95
Gmi_gbs_76591	27.18	Gmi_es01_c513_769	4.79	Gmi_es02_c17403_291	27.66	Gmi_es15_c5837_115	7.95
Gmi_es02_c631_591	27.18	Gmi_es05_c497_505	4.79	Gmi_gbs_96371	27.66	Gmi_es15_c1671_378	8.28
Gmi_ds_cc3275_96	27.57	Gmi_es15_c2918_866	5.52	Gmi_es01_c28146_550	27.66	Gmi_es02_c18066_185	8.28
Gmi_gbs_18083	32.33	Gmi_es15_c2918_866	5.52	Gmi_es_lb_8047	27.66	Gmi_es15_c4142_273	8.28
Gmi_ds_lb_6017	32.33	Gmi_es15_c2918_866	5.52	Gmi_gbs_45234	27.66	Gmi_es02_c18066_272	8.28
Gmi_es02_lrc33175_496	32.33	Gmi_es03_lrc21633_95	11.41	Gmi_es15_c12384_386	28.03	Gmi_es02_c19924_97	8.28
Gmi_es03_c9203_225	32.33	Gmi_gbs_77286	16.14	Gmi_gbs_20448	28.4	Gmi_es03_c2277_336	8.61
Gmi_es01_c262_877	32.33	Gmi_es14_c617_370	17.29	Gmi_es03_c13331_202	28.4	Gmi_es02_c1268_213	8.61
Gmi_es03_c12159_493	32.33	Gmi_es14_c7020_89	35.51	Gmi_es03_c5208_224	28.4	Gmi_es15_c16513_175	8.61
Gmi_es_cc14968_101	32.33	Gmi_gbs_50325	35.51	Gmi_es14_c16054_391	28.4	Gmi_es15_c2701_67	8.61
Gmi_es22_lrc11252_266	32.33	Gmi_es17_c3418_95	35.51	Gmi_es03_c7453_413	28.4	Gmi_es05_c8955_177	8.61
Gmi_es17_c16508_298	32.33	Gmi_es05_c15526_511	35.51	Gmi_es03_c2772_448	28.4	Gmi_es17_c4686_486	8.61
Gmi_gbs_114272	32.68	Gmi_es14_c19842_74	35.51	Gmi_gbs_13560	28.4	Gmi_ds_lb_10616	8.61
Gmi_es01_c9085_780	33.07	Gmi_gbs_6367	36.95	Gmi_es02_c12285_761	28.4	Gmi_es_lb_11852	8.61
Gmi_es15_c7879_555	34.03	Gmi_es03_lrc9679_178	36.95	Gmi_es05_c22428_236	28.74	Gmi_es13_c2873_647	8.61
Gmi_es22_c3052_382	34.96	Gmi_ds_lb_4000	37.3	Gmi_es22_c8130_418	29.53	Gmi_es17_c9765_99	8.61
		Gmi_es01_c1635_353	37.3	Gmi_ds_cc9305_53	29.53	Gmi_gbs_9360	8.61
		Gmi_es05_c2452_649	37.3	Gmi_es02_c11775_206	29.53	Gmi_es05_c10342_81	8.61

Linkage Group 9		Linkage Group 10		Linkage Group 11		Linkage Group 12	
Marker Name	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**
		Gmi_es01_c8470_599	37.3	Gmi_es15_c6098_227	29.53	Gmi_es03_c10695_533	8.61
		Gmi_es_cc8700_285	37.3	Gmi_es15_c4675_465	29.53	Gmi_es17_c4128_744	8.61
		Gmi_es05_c8599_963	37.3	Gmi_es02_c1532_592	29.53	Gmi_es14_c8352_658	8.61
		Gmi_es_cc4978_509	37.3	Gmi_es05_c17724_98	29.53	Gmi_es01_c10162_242	8.61
		Gmi_es03_c10621_322	38.42	Gmi_es18_c3776_562	29.53	Gmi_es15_c5371_294	8.61
		Gmi_es14_c7737_88	38.78	Gmi_es08_c6067_179	29.53		
		Gmi_es17_c20641_316	50.85	Gmi_es01_c16302_758	29.53		
		Gmi_es14_c18293_91	50.85	Gmi_es03_c1168_855	29.53		
		Gmi_es02_c37525_294	50.85	Gmi_es03_c3011_446	30.3		
		Gmi_ds_cc2679_57	50.85	Gmi_es15_c15279_258	31.16		
		Gmi_gbs_73795	50.85	Gmi_es05_c2343_456	31.57		
		Gmi_es14_c967_122	50.85	Gmi_es02_c23736_166	31.57		
				Gmi_es17_c2417_887	31.57		
				Gmi_es22_c9552_420	31.57		
				Gmi_es03_c95_413	31.57		
				Gmi_es_lb_11175	31.57		
				Gmi_ds_a3_74_292	31.57		

Linkage Group 13		Linkage Group 14		Linkage Group 15		Linkage Group 16	
Marker Name	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**
Gmi_es05_c19004_333	0	Gmi_es17_c11370_658	0	Gmi_es03_c12087_253	0	Gmi_es_lb_8613	0
Gmi_es05_c11381_538	0	Gmi_es17_c3477_85	7.32	Gmi_gbs_109117	6.13	Gmi_es14_c78_442	0.36
Gmi_es14_c6932_255	15.53	Gmi_es15_c9667_138	10.63	Gmi_es01_c12905_677	6.84	Gmi_es22_c4252_400	0.36
Gmi_es03_c4826_298	16.27	Gmi_es22_c567_627	10.63	Gmi_es01_c14139_498	6.84	Gmi_es01_c14650_200	0.36
Gmi_es17_c11262_129	16.27	Gmi_es02_c15898_126	14.64	Gmi_gbs_24059	6.84	Gmi_ds_lb_6955	2.47
Gmi_es15_c7196_299	16.27	Gmi_es14_c940_534	16.95	Gmi_es01_c3899_470	6.84	Gmi_ds_a3_468_401	22.39
Gmi_es_lb_12096	16.27	Gmi_es22_c8670_78	16.95	Gmi_es01_c8337_431	6.84	Gmi_gbs_112922	22.39
Gmi_es15_c784_396	16.63	Gmi_es15_c6114_189	16.95	Gmi_gbs_6566	7.19	Gmi_es01_c11484_64	22.39
Gmi_ds_lb_8648	16.99	Gmi_es17_c5367_259	16.95	Gmi_es17_c2308_1026	7.19	Gmi_gbs_14638	22.39
Gmi_gbs_84842	16.99	Gmi_es15_c6130_326	17.29	Gmi_ds_cc9448_443	7.19	Gmi_gbs_14638	22.39
Gmi_es14_c905_71	19.92	Gmi_es15_c713_848	17.29	Gmi_gbs_3516	8.6	Gmi_gbs_14638	22.39
Gmi_es14_c905_429	19.92	Gmi_es15_c2041_605	17.29	Gmi_gbs_61050	8.6	Gmi_es02_c4114_760	27.21
Gmi_es15_c3133_51	21.41	Gmi_ds_lb_8383	17.29	Gmi_gbs_77957	10.04	Gmi_ds_lb_9376	30.09
Gmi_es17_c16402_247	22.51	Gmi_es03_c9245_234	17.29	Gmi_es14_c6249_380	35.37	Gmi_es01_c23169_232	30.09
Gmi_es_cc9362_188	22.51	Gmi_es14_c11471_199	17.29	Gmi_gbs_95417	35.37		
Gmi_es14_c7167_253	32.3	Gmi_es_lb_4286	17.29	Gmi_es14_c8194_77	35.37		
Gmi_es14_c16196_221	32.3	Gmi_es22_c4367_376	17.29	Gmi_es01_c9549_89	35.73		
		Gmi_es11_c397_1001	17.29	Gmi_gbs_112713	35.73		
		Gmi_es03_c15150_160	17.29	Gmi_gbs_73388	35.73		
		Gmi_gbs_27180	17.29	Gmi_es15_c5315_156	36.07		
		Gmi_es_cc8503_89	17.29	Gmi_es_lb_5270	36.42		
		Gmi_es02_c12776_148	17.29	Gmi_es02_c22155_716	36.42		
		Gmi_es02_c23814_94	17.29	Gmi_es11_c12575_587	36.81		
		Gmi_es01_lrc22977_595	17.29	Gmi_es17_c3397_167	37.19		
		Gmi_es17_c9625_419	17.29	Gmi_es14_c2965_193	37.19		
		Gmi_es02_c16349_294	19.13	Gmi_es_lb_9817	37.19		
		Gmi_es02_c16349_294	19.13	Gmi_es02_c4277_710	37.9		
		Gmi_es02_c19630_126	21.66	Gmi_es01_c6996_570	50.08		
		Gmi_es02_c19630_126	21.66	Gmi_es15_c4877_111	51.96		

Linkage Group 13		Linkage Group 14		Linkage Group 15		Linkage Group 16	
Marker Name	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**
		Gmi_es02_c19630_126	21.66	Gmi_es15_c482_113	51.96		
		Gmi_es14_c1298_233	25.97	Gmi_es03_lrc9889_122	51.96		
		Gmi_es14_c2927_401	26.33				
		Gmi_es14_c12956_103	27.04				
		Gmi_es05_c1760_188	40.28				
		Gmi_es_lb_8449	40.28				
		Gmi_es17_c18708_336	40.28				
		Gmi_gbs_11780	40.28				
		Gmi_es01_c3919_84	40.28				
		Gmi_es02_c12535_326	40.28				
		Gmi_es05_c10905_124	40.28				
		Gmi_es05_c11419_658	40.28				
		Gmi_es17_c4526_333	40.28				
		Gmi_es17_c4526_333	40.28				
		Gmi_ds_cc4430_239	42.92				
		Gmi_es15_c7380_296	43.29				
		Gmi_es_lb_3611	43.67				
		Gmi_es02_c18392_445	43.67				
		Gmi_es_lb_11832	44.36				
		Gmi_es14_c502_747	44.36				
		Gmi_es_lb_2973	44.72				
		Gmi_gbs_80856	45.46				
		Gmi_es22_c5531_401	45.46				
		Gmi_es15_c14992_307	45.46				
		Gmi_es05_c8916_635	45.46				
		Gmi_es05_c9640_317	45.82				
		Gmi_es17_lrc7334_312	45.82				
		Gmi_es17_c4241_356	45.82				
		Gmi_es14_lrc18713_286	46.2				
		Gmi_es15_c6120_507	46.2				

Linkage Group 13		Linkage Group 14		Linkage Group 15		Linkage Group 16	
Marker Name	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**
		Gmi_es17_c2063_243	46.2				
		Gmi_es05_c12111_455	46.2				
		Gmi_es14_c10289_533	46.2				
		Gmi_es01_c16941_51	46.2				
		Gmi_gbs_60858	46.91				
		Gmi_es17_c2122_619	46.91				

Linkage Group 17		Linkage Group 18		Linkage Group 19		Linkage Group 20	
Marker Name	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**
Gmi_ds_lb_6090	0	Gmi_es02_c6368_605	0	Gmi_es03_c3636_522	0	Gmi_es01_c14622_328	0
Gmi_es01_c14131_316	24.38	Gmi_es01_c13467_233	7.6	Gmi_es01_c27997_135	0	Gmi_es02_c19247_238	0
Gmi_es22_c3090_150	24.38	Gmi_es02_c28832_308	7.6	Gmi_es05_c14960_147	0		
Gmi_es01_c14131_173	24.38	Gmi_es22_c928_79	7.6	Gmi_es03_c1994_232	0		
		Gmi_es02_lrc17781_234	7.6	Gmi_es02_c16824_373	0		
		Gmi_gbs_18040	7.6	Gmi_es05_c22829_277	0.68		
		Gmi_es02_c13482_582	7.6	Gmi_es14_c1948_243	0.68		
		Gmi_es14_c10878_573	7.6	Gmi_gbs_81142	0.68		
		Gmi_gbs_35276	7.95	Gmi_es02_c32043_171	0.68		
		Gmi_es02_c3604_154	7.95	Gmi_es05_c13617_330	0.68		
		Gmi_es03_c16912_416	7.95	Gmi_gbs_96110	0.68		
		Gmi_es14_c19327_378	7.95	Gmi_es14_c5853_571	1.38		
		Gmi_gbs_91128	7.95	Gmi_es02_lrc14207_485	2.08		
		Gmi_es14_c11870_550	7.95	Gmi_gbs_112943	2.08		
		Gmi_gbs_5003	7.95	Gmi_es02_c2109_540	2.08		
		Gmi_es03_c16861_126	24.43	Gmi_gbs_14322	2.08		
		Gmi_es15_c911_221	24.43	Gmi_es22_c1549_439	2.08		
		Gmi_es05_c5968_912	24.78	Gmi_es05_lrc20957_299	2.08		
		Gmi_es15_c6229_566	24.78	Gmi_es22_c9117_426	2.08		
		Gmi_gbs_29011	25.12	Gmi_gbs_80580	2.42		
		Gmi_es05_c14023_258	25.47	Gmi_ds_lb_2054	7.16		
				Gmi_es14_c8930_208	7.16		
				Gmi_es02_lrc37108_772	7.16		
				Gmi_es05_c6752_720	9.46		
				Gmi_gbs_61527	11.47		
				Gmi_es14_c1439_83	11.47		

Linkage Group 21		Linkage Group 22		Linkage Group 23		Linkage Group 24	
Marker Name	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**
Gmi_ds_cc9493_63	0	Gmi_es01_c24478_209	0	Gmi_es02_lrc37077_735	0	Gmi_es01_c1875_317	0
Gmi_es02_c975_340	2.35	Gmi_es05_c8624_796	0	Gmi_ds_lb_2910	18.91	Gmi_es03_c5032_516	0
Gmi_es22_c7720_137	3.44	Gmi_gbs_24823	1.47	Gmi_es17_c13090_302	18.91	Gmi_gbs_78053	0.35
Gmi_es15_c7905_448	18.2	Gmi_es14_c18825_425	3.22	Gmi_es15_c2423_507	19.29	Gmi_es17_c11415_605	35.83
Gmi_es_lb_12079	20.35	Gmi_es02_c40421_169	3.22	Gmi_es02_c4957_300	19.29	Gmi_es15_c17486_204	35.83
Gmi_es22_c7981_618	20.7	Gmi_es03_c18829_125	3.97	Gmi_gbs_115834	19.29	Gmi_es14_c19666_607	35.83
Gmi_es17_c12269_176	20.7	Gmi_es01_c21904_275	5.03	Gmi_es01_c26740_209	24.42	Gmi_es22_c408_595	35.83
Gmi_es15_c367_129	21.06	Gmi_es02_c4066_165	6.81	Gmi_es03_c3354_639	24.42	Gmi_ds_lb_4073	48.25
Gmi_ds_lb_2807	21.4	Gmi_es01_c14065_159	6.81	Gmi_gbs_33046	24.42	Gmi_es22_c3162_293	48.59
Gmi_es02_c4007_487	23.33	Gmi_es03_lrc22399_424	28.94	Gmi_gbs_33046	24.42	Gmi_gbs_97971	49.63
Gmi_es02_c2959_310	23.33			Gmi_gbs_33046	24.42	Gmi_gbs_40539	50.68
Gmi_es05_c14480_201	24.42			Gmi_es05_c12282_370	29.12	Gmi_es02_c4100_1210	57.12
Gmi_es03_c5662_209	42.89			Gmi_es15_c19177_191	29.12		
Gmi_gbs_6231	43.94			Gmi_es22_c6291_426	29.12		
Gmi_gbs_94275	44.65			Gmi_gbs_52130	29.12		
Gmi_es02_c8277_506	45.35			Gmi_es14_c10334_407	29.12		
Gmi_es03_c2622_120	45.35			Gmi_es01_c19122_222	29.49		
Gmi_ds_lb_1859	45.35			Gmi_gbs_111661	30.72		
Gmi_es15_c6639_318	47.21			Gmi_gbs_75415	31.86		
				Gmi_es02_c2875_1066	56.62		
				Gmi_es02_c2961_257	84.48		
				Gmi_es15_c5368_259	88.41		
				Gmi_es05_c20848_84	88.41		
				Gmi_es02_c1643_792	88.41		
				Gmi_es03_c16835_129	88.41		
				Gmi_es17_c12516_818	88.41		
				Gmi_es05_c9397_421	88.41		
				Gmi_ds_cc4394_195	88.41		
				Gmi_es05_c15948_428	88.41		
				Gmi_es22_c16302_276	88.41		

Linkage Group 21		Linkage Group 22		Linkage Group 23		Linkage Group 24	
Marker Name	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**
				Gmi_es05_c10300_165	88.41		
				Gmi_es15_c1060_702	91.53		

Linkage Group 25		Linkage Group 26		Linkage Group 27		Linkage Group 28	
Marker Name	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**
Gmi_es03_c1189_805	0	Gmi_ds_lb_4162	0	Gmi_es14_c2320_26	0	Gmi_es02_c3577_672	0
Gmi_gbs_89713	0	Gmi_es14_c9604_176	0.34	Gmi_gbs_81139	0	Gmi_es02_c29912_249	0.34
Gmi_es03_lrc9098_528	0	Gmi_es01_c9092_330	0.34	Gmi_es17_c899_571	0	Gmi_es14_c2441_215	2.09
Gmi_es07_c4037_548	7.13	Gmi_es15_c316_208	0.7	Gmi_ds_lb_3936	0	Gmi_es03_c20076_383	2.09
Gmi_es15_c12291_689	7.13	Gmi_es02_c15583_373	1.77	Gmi_es22_c1298_437	0	Gmi_es22_c659_767	8.67
Gmi_es17_c4716_700	7.49	Gmi_es18_c503_513	1.77			Gmi_es17_c5464_219	10.43
Gmi_es17_lrc20172_521	7.49	Gmi_es14_c19374_365	1.77			Gmi_es15_c5457_190	10.43
Gmi_es17_c9953_261	7.49	Gmi_es_cc8927_168	2.13				
Gmi_es02_c2714_373	8.51	Gmi_es_cc9916_519	8.69				
Gmi_es17_c8805_454	9.91	Gmi_es22_c12811_64	8.69				
Gmi_es02_c15089_196	9.91						
Gmi_gbs_58632	9.91						
Gmi_es05_c4270_561	10.25						
Gmi_es05_c4270_561	10.25						
Gmi_gbs_114111	13.74						
Gmi_es22_c19565_289	15.71						
Gmi_es15_c18028_289	15.71						
Gmi_ds_lb_7757	15.71						
Gmi_es17_c5197_503	15.71						

Linkage Group 29		Linkage Group 30		Linkage Group 31		Linkage Group 32	
Marker Name	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**	**Marker Name**	**Pos**
Gmi_es02_c8186_443	0	Gmi_es15_c13619_180	0	Gmi_es01_c4452_325	0	Gmi_es05_c1138_484	0
Gmi_es01_c4084_320	2.65	Gmi_es01_lrc9332_388	0	Gmi_ds_opt_17694_374	5.58	Gmi_es15_c2358_404	0.69
Gmi_es02_c13827_987	14.72	Gmi_gbs_2670	3.61	Gmi_ds_lb_6395	6.63	Gmi_gbs_92155	0.69
Gmi_es14_c4050_361	14.72	Gmi_es17_c3973_587	3.61	Gmi_es17_c16130_411	19.16	Gmi_es17_c18602_497	4.55
		Gmi_es22_c7787_334	12.88	Gmi_es17_c24_198	19.52	Gmi_gbs_95069	5.29
				Gmi_es_lb_11728	19.52	Gmi_es22_lrc15031_170	8.87
				Gmi_es05_c10573_58	19.86		
				Gmi_es02_c15228_655	29.48		
				Gmi_gbs_60431	29.48		

Linkage Group 33		Linkage Group 34	
Marker Name	**Pos**	**Marker Name**	**Pos**
Gmi_ds_lb_6342	0	Gmi_es15_c6435_299	0
Gmi_es17_c3370_293	11.4	Gmi_es01_c9384_567	19.15
Gmi_es02_c2554_426	19.81	Gmi_es02_lrc13826_351	43.32
Gmi_es17_c12067_507	25.16	Gmi_es22_c17932_519	49.7

Appendix D

CDC Dancer (OT344 x W90279) Pedigree: Seven Generations

1	2	3	4	5	6	7
					unknown	unknown
				unknown		unknown
					unknown	unknown
			unknown			unknown
					unknown	unknown
				unknown		unknown
					unknown	unknown
		CI964P2-R4				unknown
					unknown	unknown
				unknown		unknown
					unknown	unknown
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					unknown	unknown
	OT235					unknown
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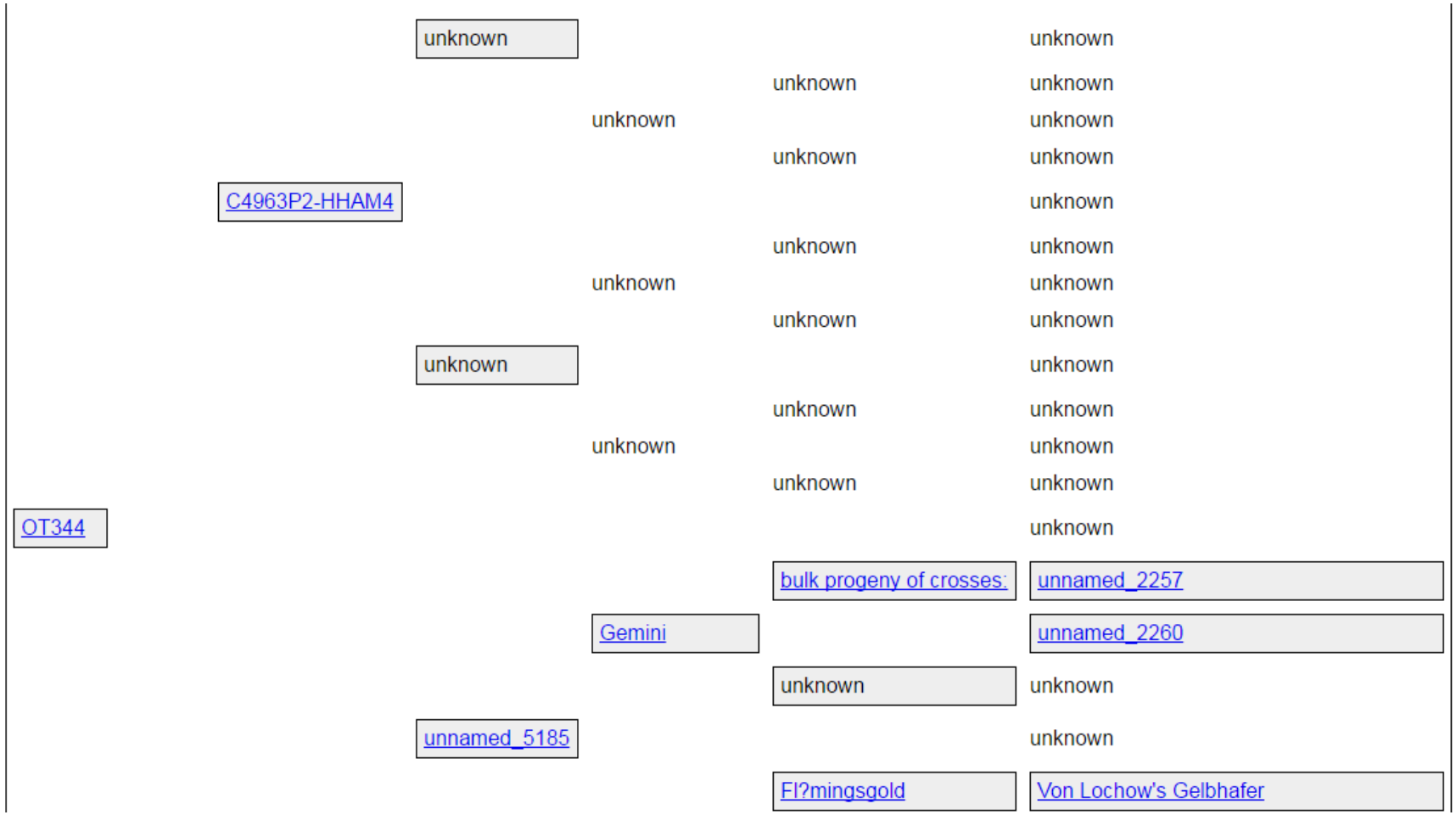
					unknown	unknown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unknown
unknown
unknown
unknown
unknown
C4963P2-HHAM4
unknown
unknown
unknown
unknown
unknown
unknown
unknown
unknown
unknown
unknown
unknown
unknown
unknown
OT344
unknown
bulk progeny of crosses:
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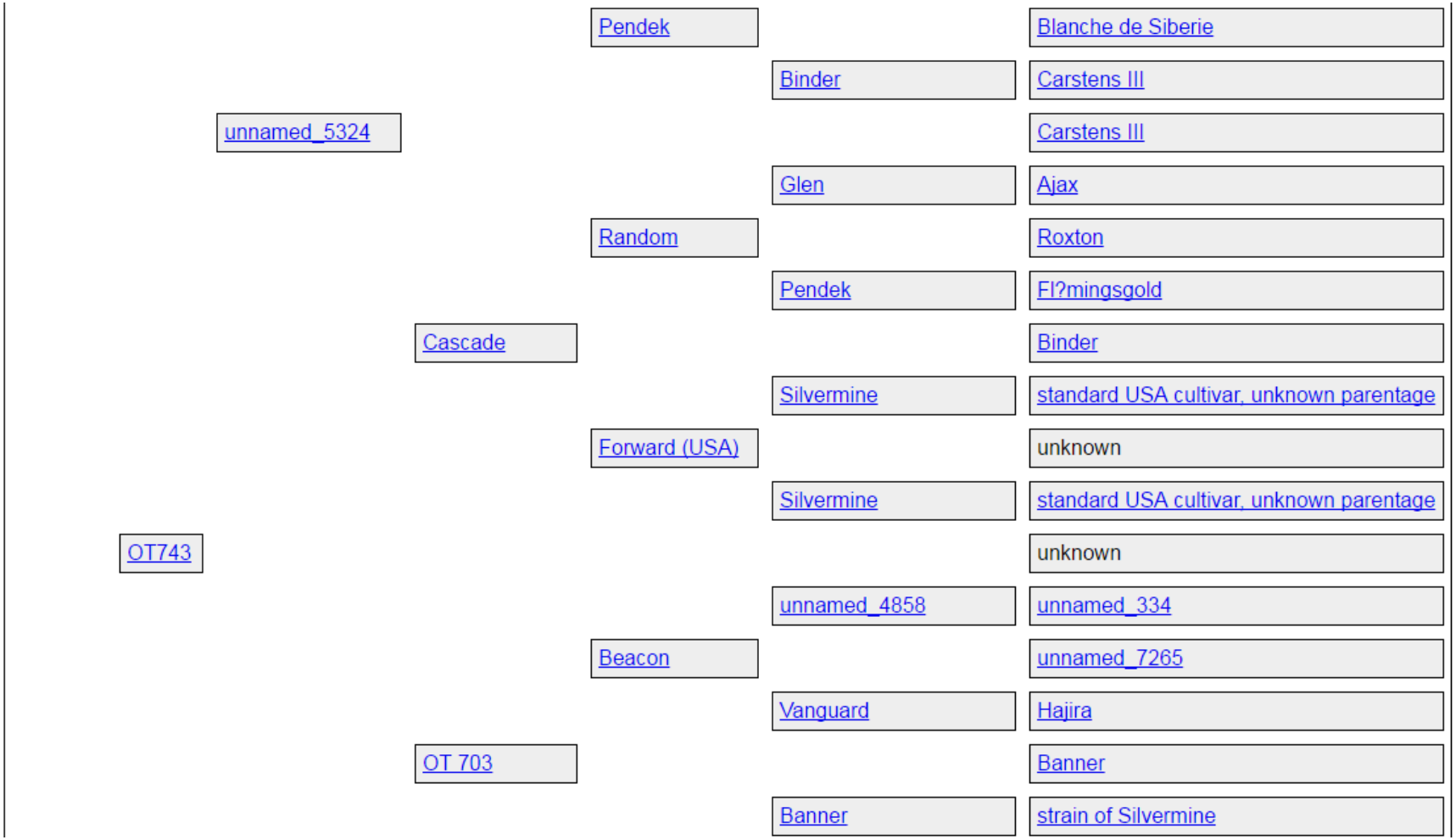
Gemini
unnamed_2260
unknown
unknown
unnamed_5185
unknown
Fl?mingsgold
Von Lochow's Gelbhafer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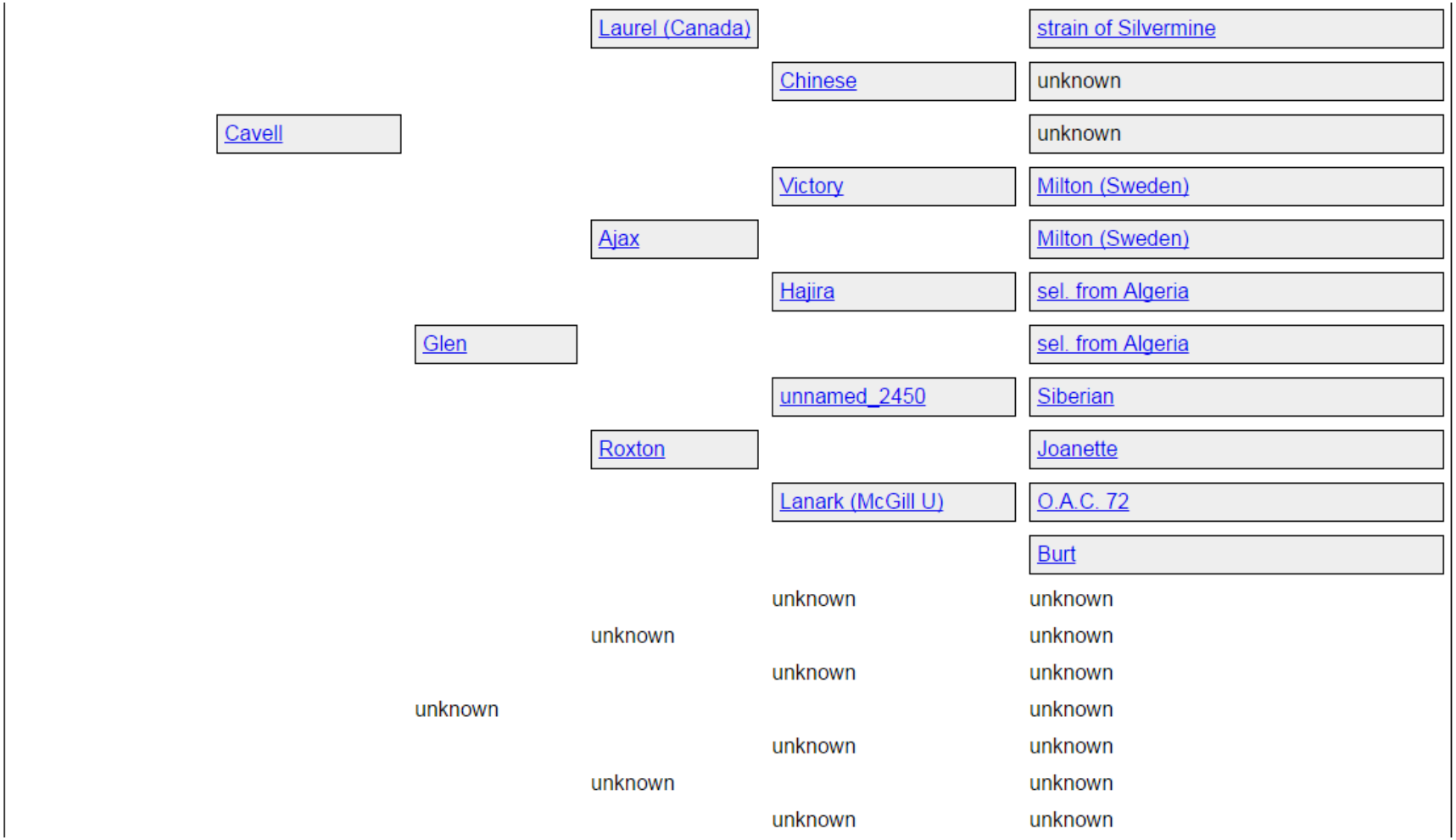
Blanche de Siberie
Binder
Carstens III
unnamed_5324
Carstens III
Glen
Ajax
Random
Roxton
Pendek
Fl?mingsgold
Cascade
Binder
Silvermine
standard USA cultivar, unknown parentage
Forward (USA)
unknown
Silvermine
standard USA cultivar, unknown parentage
OT743
unknown
unnamed_4858
unnamed_334
Beacon
unnamed_7265
Vanguard
Hajira
OT 703
Banner
Banner
strain of Silvermine


Laurel (Canada)
strain of Silvermine
Chinese
unknown
Cavell
unknown
Victory
Milton (Sweden)
Ajax
Milton (Sweden)
Hajira
sel. from Algeria
Glen
sel. from Algeria
unnamed_2450
Siberian
Roxton
Joanette
Lanark (McGill U)
O.A.C. 72
Burt
unknown
unknown
unknown
unknown
unknown
unknown
unknown
unknown
unknown
unknown
unknown
unknown

	unknown			unknown
			unknown	unknown
		unknown		unknown
			unknown	unknown
	unknown			unknown
			unknown	unknown
		unknown		unknown
			unknown	unknown
Konrad				unknown
			unknown	unknown
		unknown		unknown
			unknown	unknown
	unknown			unknown
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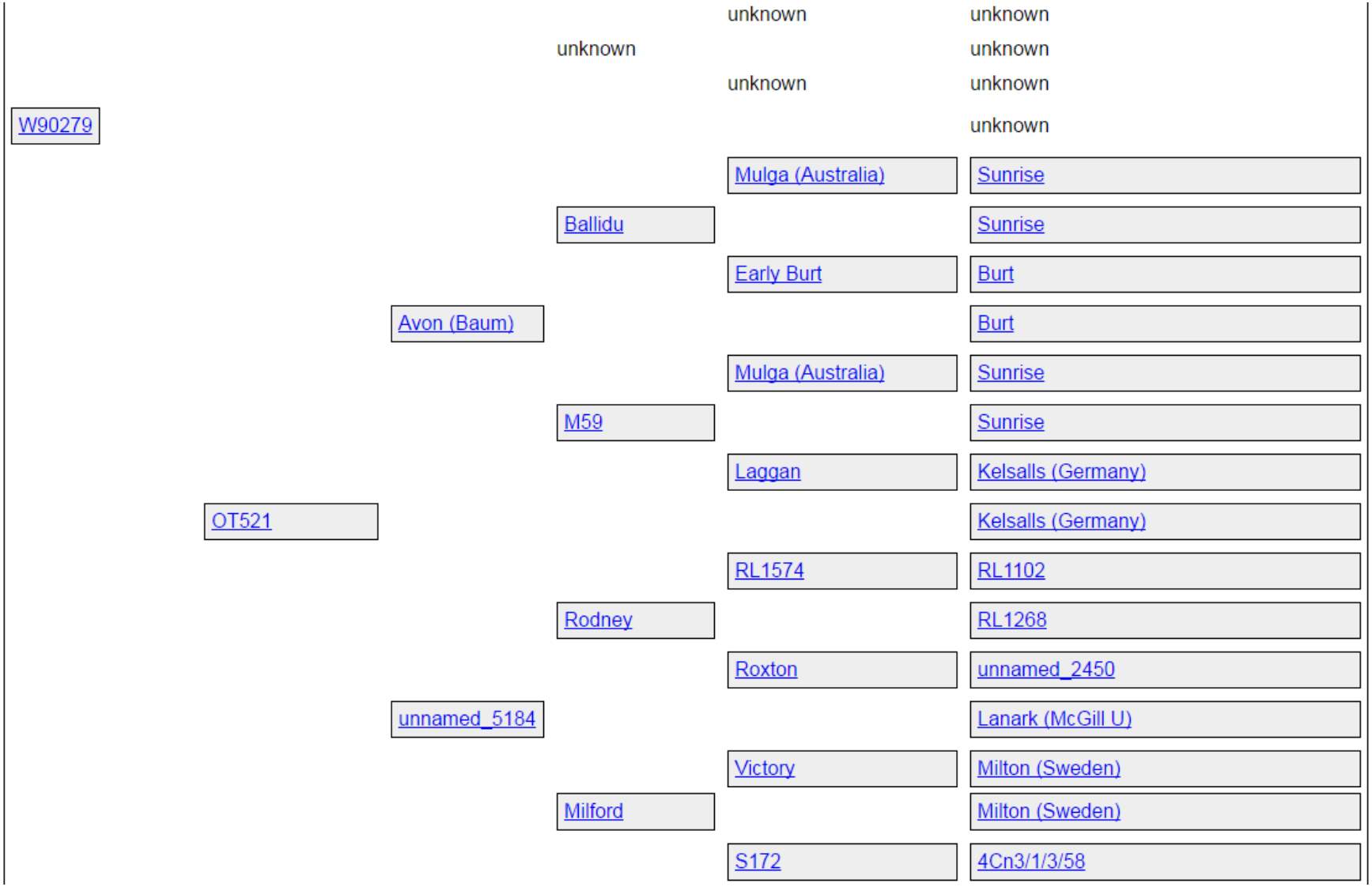
W90279
OT521
Avon (Baum)
unnamed_5184
Ballidu
M59
Rodney
Milford
unknown
unknown
unknown
unknown
unknown
unknown
unknown
unknown
Mulga (Australia)
Early Burt
Mulga (Australia)
Laggan
RL1574
Roxton
Victory
S172
Sunrise
Sunrise
Burt
Burt
Sunrise
Sunrise
Kelsalls (Germany)
Kelsalls (Germany)
RL1102
RL1268
unnamed_2450
Lanark (McGill U)
Milton (Sweden)
Milton (Sweden)
4Cn3/1/3/58

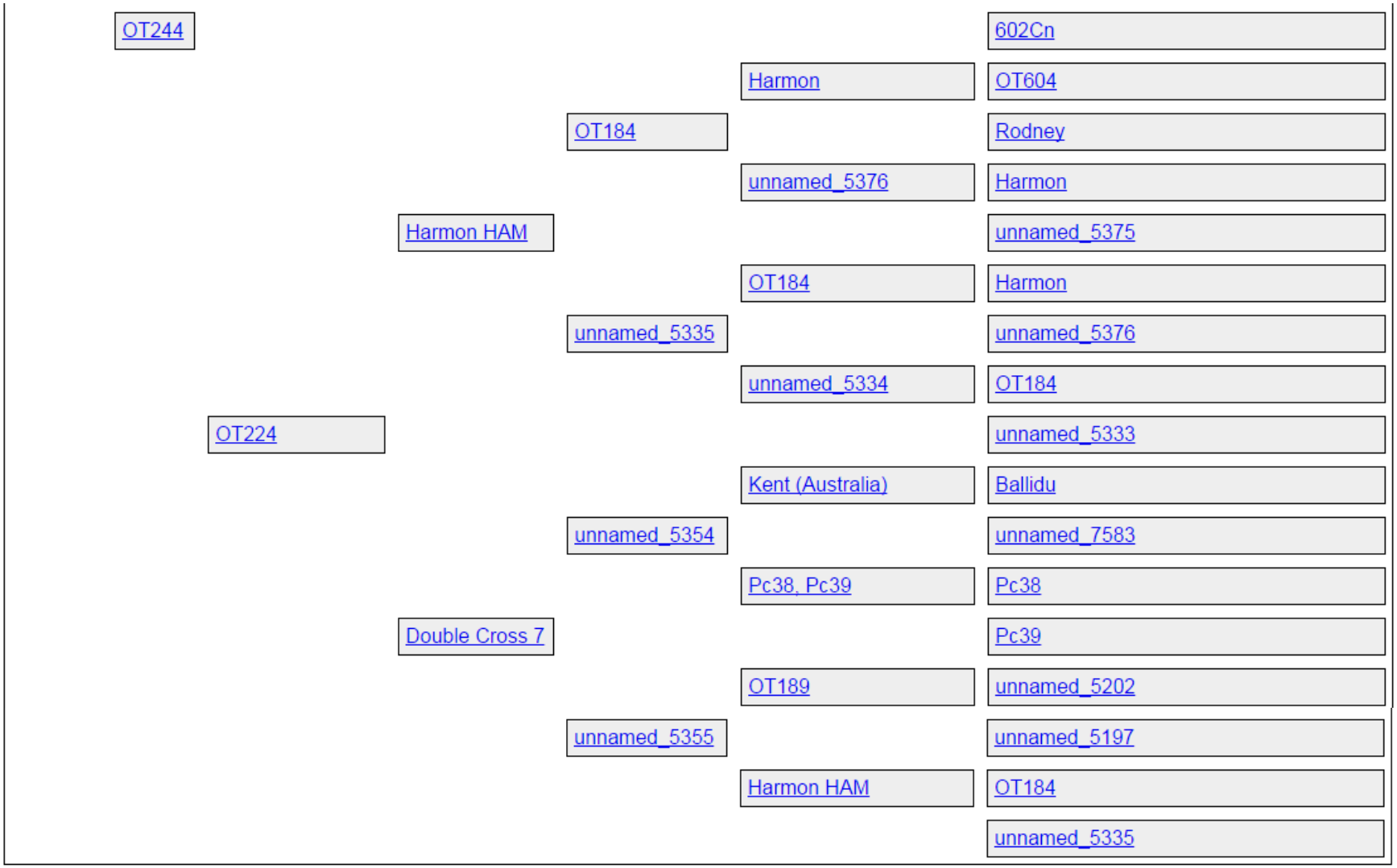

Figure D1. Pedigree of 'CDC Dancer' from Pedigrees of Oat Lines (POOL). Possible sources of loose smut resistance include 'Harmon HAM' and 'Beacon' that descended from 'Black Mesdag' (not shown), 'Burt' that is a descendant of 'Red Rustproof' (not shown), and 'Siberian' and 'O.A.C. 72', which are synonyms of 'Sibiryak'.

Appendix E
Yield Trial Location Information

Table E1. Detailed agronomic and site information for the 12 locations used to study the effect of loose smut resistance on yield of oat.

Descriptor	Brandon, MB	Codette, SK	Fargo, ND, USA	Goodale, SK
Longitude	49°51'48.4632" N	53°16'52"N	n/a	52°03’33.9”N
Latitude	99°54'42.5484" W	103°52'0.58"W	n/a	106°29’08.5”W
Elevation	490m	372m	n/a	486m
Soil type	thin black	grey luvisol	n/a	orthic dark brown, loam
Planting date	20-May-15	May-29	n/a	May-14
Harvest Date	25-Aug-15	Sep-30	n/a	Aug-31
Plot size	4.5m2	6.858m2	n/a	4.32m2
Row Spacing	7.09" (18 cm)	8" (20.32cm)	n/a	16" (40.64cm)
Seeding density	33.33g/m2 (1400 seeds/plot = 311 seeds/m2)	80g/plot	1400 seeds/plot	1400 seeds/plot should equal 325 seeds/m2, plant density 215/m2
Fertilizer formulation	46-0-0, 43-11-52	N:46-0-0-0, P:11-52-0-0, K:0-0-62-0, S:21-0-0-24	n/a	28-23-0-0
Fertilizer rate	243 kg/ha, 43 kg/ha	N 80.5lbs/ac, P 30lbs/ac, K 30 lbs/ac, S 7 lbs/ac	n/a	50lbs/acre
Herbicide formulation	Startup, MCPA 500 amine, Lorox L	Prestige XC A &B	n/a	Refine SG, MCPA Ester 600
Herbicide rate	Startup 1L/acre, MCPA 0.4L/acre; Lorox L 0.22L/acre, MCPA 0.45L/acre	Prestige XC A: 170mL/acre, Prestige XC B: 800mL/acre	n/a	12g/acre, 0.42L/acre
Fungicide formulation	Folicur 3.6, Headline EC	Folicur 250EW, Quilt	n/a	Folicur 250 EW, Quilt
Fungicide rate	Folicur 60mL/acre; Headline 160mL/acre	Folicur 75mL/acre; Quilt 304mL/acre	n/a	Folicur 150mL/acre; Quilt 405mL/acre

Descriptor	Kamsack, SK	Kelburn, MB	Kernen, SK	Lacombe, AB
Longitude	51°31'30.5"N	49 47'37.7"N	52°09’06.7”N	52°27’23”N
Latitude	102°04'42.3"W	97 14'70.1"W	106°31’41.7”W	113°44’39.5”W
Elevation	490m	239m	486m	850m
Soil type	sandy chernozem	clay	orthic dark brown, clay-clay loam	black
Planting date	May-15	Apr-29	May-16	May-17
Harvest Date	Sep-15	Aug-16	Sep-04	Sep-22
Plot size	4.28m2	9m2	4.32m2	5m2
Row Spacing	7"(17.8cm)	7"(17.8cm)	16" (40.64cm)	n/a
Seeding density	1968 seeds/plot (460 seeds/m2)	1980 seeds/plot (220 seeds/m2)	1400 seeds/plot, plant density 215/m2	1200seeds/5m2
Fertilizer formulation	46-0-0 urea	11-52-0, 46-0-0	28-23-0-0	n/a
Fertilizer rate	180lbs/acre	65lbs/acre with seed, 120lbs/acre broadcast	50lbs/acre	n/a
Herbicide formulation	Buctril	Curtail M	Buctril M	n/a
Herbicide rate	400ml/ac	0.61L/ac	0.4L/ac	n/a
Fungicide formulation	n/a	none applied	Folicur 250 EW	n/a
Fungicide rate	n/a	none applied	Folicur 150mL/acre; Quilt 405mL/acre	n/a

Descriptor	Melfort, SK	Ottawa, ON	Quebec City, QC	Saskatoon, SK
Longitude	52°48.823'N	45°25'N	45°36’09.1”N	52°08’25.1”N
Latitude	104°36.809'W	75°41'W	72°33’03.2”W	106°36’50.7”W
Elevation	469m	70m	32m	486m
Soil type	black chernozem	clay loam	clay loam	orthic dark brown, silty loam-clay
Planting date	May-22		4-May-15	May-11
Harvest Date	Sep-10		20-Aug-15	Sep-02
Plot size	4.32m2	3.8m2	5m2	4.32m2
Row Spacing	16" (40.64cm)	7"(17.8cm)	6.25" (15.88cm)	16" (40.64cm)
Seeding density	1400 seeds/plot, plant density 215/m2	300seeds/m2 (1400 seeds/plot)	375/m2 (2250 seeds/plot)	1400 seeds/plot, plant density 215/m2
Fertilizer formulation	none applied	N	27.2-13.6-10.2	28-23-0-0
Fertilizer rate	none applied	50lbs/acre	146.9kg/ha	50lbs/acre
Herbicide formulation	Prestige A&B	Buctril M	Logic M	none applied
Herbicide rate	Prestige A 0.4L/ac, Prestige B 2.0 L/ha		1.25L/ha	none applied
Fungicide formulation	Folicur	Folicur, Quilt	Folicur, Quilt	Folicur 250 EW, Quilt
Fungicide rate	150mL/ha	Folicur 175mL/ha; Quilt 750mL/ha	Folicur 146mL/ha; Quilt 1000mL/ha	Folicur 150mL/acre; Quilt 405mL/acre

Appendix F
Field Trial Yield Data of Near Isogenic Lines

Table F1. Yield data of the two near isogenic lines grown at the 12 locations used to study the effect of loose smut resistance on yield.

	NIL Yield (kg/ha)			NIL Yield (kg/ha)	
Location	T-904-01-151 (Resistant)	T-904-01-163 (Susceptible)	**Location**	T-904-01-151 (Resistant)	T-904-01-163 (Susceptible)
Brandon, MB	7814	7748	**Codette, SK**	1930	3077
	7093	7756		2091	2470
	8026	6549		1296	932
Avg.	7644	7351	**Avg.**	1772	2160
SD	489	694	**SD**	420	1106
Fargo, ND	2720	3500	**Goodale, SK**	5027	4788
	3529	4058		5093	4919
	2544	3267		4835	—
Avg.	2931	3608	**Avg.**	4985	4854
SD	525	406	**SD**	134	93
Kamsack, SK	2769	2371	**Kelburn, MB**	5343	5479
	2580	2830		5433	5467
	—	—		5189	4831
Avg.	2675	2601	**Avg.**	5322	5259
SD	134	325	**SD**	123	371
Kernen, SK	4151	4163	**Melfort, SK**	4613	3438
	4190	3735		5061	3427
	4720	3895		5061	3079
Avg.	4354	3931	**Avg.**	4912	3315
SD	318	216	**SD**	259	204
Ottawa, ON	4482	4850	**Quebec City, QC**	3761	5446
	4692	4878		4127	4569
	3920	4460		4312	4530
Avg.	4365	4729	**Avg.**	4067	4848
SD	400	234	**SD**	280	518
Saskatoon, SK	4747	4795			
	5104	4550			
	5501	5346			
Avg.	5117	4897			
SD	377	408			